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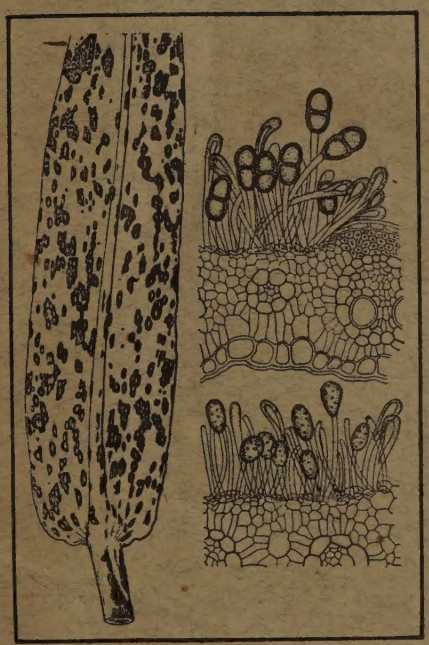

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FUNGI IN THE NEST OF ODONTOTERMES OBESUS

By

B. K. BAKSHI

(Accepted for publication April, 1951)

Termites are known to cultivate fungi inside their mounds. Petch (1913) has given a resume of the termite fungi described from the different parts of the world. Bose (1923) stated that the only fungus that appeared, though rarely, on living mounds of *Odontotermes obesus* in Barkuda was an agaric, *Collybia albuminosa* (Berk.) Petch with its long rooting stalk going down into the combs. Different forms of *Xylaria*, all representing *X. nigripes*, were found only in abandoned termite nests. A reference is made by Bose to "a thick white mycelial mass of sessile spheres which in the course of a week, turned green like green moulds on decomposed wood. The mass remained the same colour throughout, and eventually produced a number of rounded sporangia consisting of green spores." It is not clear as to what has been referred to by Bose in his statement. In view of the fact that the spheres form the main component of the fungus-garden cultivated by termites, I have described them as I have seen in the termite mounds of *Odontotermes obesus* in New Forest, Dehra Dun.

Pl. 1, figs. 1 and 2 show the mycelium and spheres within the comb of *Odontotermes obesus*. Their essential features are described below and for details the work of Petch (1906) may be referred to. Hyphae are usually abundunt, cottony, thin-walled or slightly thick-walled (Text-fig. 5), hyaline, septate (simple), 2—5 μ broad. Chlamydospores develop rarely (Text-fig. 4) as was also observed by Ciferri (1935). They are hyaline, thin-walled, 11—13 $\times$ 8—9 μ. Some of the hyphae unite to form spheres which, in young and mature stages, are milk-white in colour. White shiny granules are seen on the surface when they are magnified under a hand lens and these granules separate out easily when teased. A young sphere has a short stalk which swells up into a head, the latter becoming spherical with age. A mature sphere is 0.5—0.8 mm. in diameter. The stalk consists of hyphae longitudinally united and these continue into the swollen head where hyphae branch in a penicillated fashion ; the ultimate branches bear conidia singly or in chains (Text-fig. 1). Conidia are hyaline, oval or cylindric (Text-fig. 2), 12—22 $\times$ 5—7.3 μ. These are enveloped by spherical cells (also referred to as conidia by workers) which develop from the stalk and multiply by budding (Text-fig. 3). They measure 33—110 $\times$ 30—45 μ.

Aseptic transfer of sphere into malt agar resulted in a culture where spheres developed abundantly (Pl. 1, fig. 3) and their structures are essentially the same as those found in nature. Hyphae are thin-walled, hyaline 1—4 (-6) μ broad, with simple septa (Text-fig. 6) and rarely with indications of a clamp connection (Text-fig. 7). Chlamydospores are common : they are thin-walled, hyaline, single or in chains (Text-fig. 8), 12—28 $\times$ 10—22 μ.

No other fungus was observed inside the comb. I frequently inspected numerous termite mounds in New Forest during rains in search of agarics which are said to develop from within the termite mounds, but without success. Stroma of *Xylaria nigripes* was sometimes found on the surface of deserted combs. Incubation of portions of the comb in the laboratory always yielded numerous sclerotial bodies but none of these developed perithecia. These sclerotia probably belong to *Xylaria* as stated by previous workers.

Text-figs. 1—8. *Termitosphaeria duthiei* (Berk.) Ciferri. 1, internal hyphae of the sphere showing conidia ; 2, conidia ; 3, outer spherical cells of the sphere showing budding, 4, chlamydospore on hyphae from the comb ; 5, hyphae from the comb ; 6, hyphae from culture ; 7, hyphae from culture showing indication of a clamp connection ; 8, chlamydospores from culture.
1, 4, 5, 6, 7, 8, × 1,300 ; 2, 3, × 500.

The spheres have been named by Berkeley as *Aegerita duthiei* which belongs to Moniliales. Since *Aegerita* is characterised by solitary (never catenulate) monomorphous conidia, Ciferri (1935) transferred the species to a new genus, *Termitosphaeria*, also under Moniliales, and re-named it as *Termitosphaeria duthiei*. This fungus has been shown by Cheo (1942) to be a conidial stage of the agaric, *Collybia albuminosa*, which develops from termite mounds. Heim (1940) placed all agarics that develop from termite mounds under *Termitomyces* which is based on *Schulzeria striata* Beeli.

The growth of *Termitosphaeria* on inhabited combs of termites and that of *Xylaria* once the combs are deserted are constant features. Both the fungi are believed to be simultaneously present in the comb. Petch (1906) stated that under natural conditions the termites keep down *Xylaria* by continuous weeding and promote the growth of the agaric especially the white conidial spheres.

The significance of the fungus-gardens in termite nests is not clearly understood. Presence of conidia in the alimentary canal of termites supports the view that they are sometimes eaten by termites. Luscher (1951) observed that the spheres are eaten so rarely that they scarcely play a large part in the nutrition of termites. He observed that humidity and temperature in the fungus-garden area were higher than elsewhere and concluded that fungus-gardens play a large part in conditioning the micro-climate inside termite nests.

My sincere thanks are due to Dr. K. Bagchee for his interest in the work and to the Entomologist of this Institute for supplying me materials used in the present study.

MYCOLOGY BRANCH
FOREST RESEARCH INSTITUTE
DEHRA DUN, INDIA

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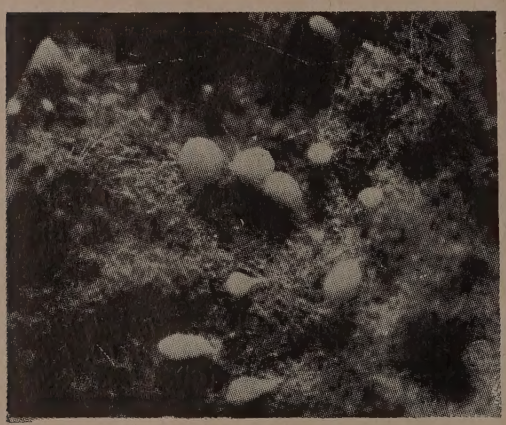

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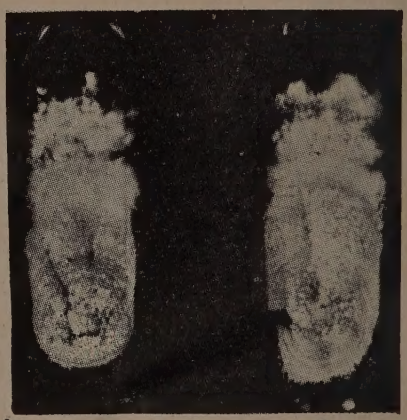

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Explanation of Plate

Fig. 1. A comb of *Odontotermes obsesus* showing white conidial spheres of *Termitosphaeria duthiei* (Berk.) Ciferri (X 1).

Fig. 2. Magnified view of fig. 1 showing hyphae and spheres (X 25).

Fig. 3. Culture of *T. duthiei* on malt agar showing numerous conidial spheres (X 1).

SCLEROGRAPHIUM ATERRIMUM BERKELEY

By

S. J. HUGHES

(Accepted for publication April 23, 1951)

Berkeley (1854) published his genus *Sclerographium* for the single species *S. aterrimum* Berk. described 'on the under side of the leaves of some species of *Indigofera*, probably *I. atropurpurea*. India.' As far as I am aware it has never been found since ; the type collection preserved in Herb. R.B.G. Kew, which indicates that the collection was made in 'Southern India', has been redescribed by v. Hohnel (1910) and the redescription given below of this type species differs little from his, although he did not describe in detail the method of conidial insertion. Because the fungus does not appear to have been illustrated since 1854 some figures are included here (Fig. 1).

Fig. 1. *Sclerographium aterrimum*: from the type collection in the Herb. R.B.G. Kew; *A*, synnema; *B*, distal ends of synnematous hyphae and conidia; ×500, except *A*, which has a scale provided.

Description of Sclerographium aterrimum

The *colonies* are hypophyllous and cover the whole surface of the leaflets: they are visible to the naked eye because of the conspicuous

black, thread-like synnemata ; there is no discolouration of the leaflets.

The *visible mycelium* is superficial, inconspicuous, and composed of very scanty, hyaline to subhyaline, branched, smooth-walled, septate hyphae 2 to 3μ wide ; the hyphae develop brown colour near a synnema. No sections were cut to determine whether hyphae penetrate the tissues of the leaflet.

The *synnemata* are all flat on the surface of the leaflets but it is possibly safe to assume that they were erect in the fresh material. They arise singly and are not crowded ; they are black, straight or slightly curved, 600 to 1000μ long with a spreading base above which they are 20 to 28μ wide, narrowing subulately to 11 to 20μ just below the fertile apex ; they are composed of adherent, brown, smooth walled, unbranched, septate hyphae 2 to 3μ wide ; above, the hyphae diverge slightly outwards from the axis of the synnema and increase gradually or more or less abruptly to 4 to 6.5μ which is the width of the apical cell ; the upper one to four cells of the synnematous hyphae are coarsely warted ; up to eight conspicuous, flat-topped cylindrical or truncate pegs, on which conidia were formerly attached, have been found on the terminal cell, up to four on the penultimate cell and fewer, usually one, on the third, fourth or fifth cell from the apex.

The *conidia* are produced by the blowing out of a small area of the wall of one of the five upper cells of the synnematous hyphae but I could not determine whether they develop in any particular order. The oval stalked conidial initial is continuous and subhyaline but soon develops a basal septum, brown colour, and a warted outer wall, remains oval or becomes fusoid or more or less clavate. At maturity conidia are dry, dark drown, conspicuously warted, transversely seven-septate and one to five of the cells may develop a single longitudinal septum ; they measure 31 to 38 by 9.5 to 12μ and fall away readily from the cells that bear them with the place of their origin marked by a conspicuous peg on the synnematous hypha and by a barely perceptible scar on the conidium.

I am grateful to Miss E. M. Wakefield for permission to examine the type collection.

COMMONWEALTH MYCOLOGICAL INSTITUTE,
FERRY LANE, KEW, SURREY,
ENGLAND.

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FURTHER ADDITIONS TO THE SYNCHYTRIA OF INDIA

By

S. C. Gupta and S. Sinha

(Accepted for publication April 26, 1951)

Sixteen species of *Synchytrium* have been recorded for India by Sydow and Butler (1907 & 1912), Butler and Bisby (1931), Mhatre and Mundkur (1945), Patel, Kulkarni and Dhande (1949), and Lacy (1950) on various hosts of flowering plants. Mhatre and Mundkur (1945) have discussed the critaria used by the earlier workers for the specific determination of species of *Synchytrium* and have assumed in their own investigation 'the specificity of a species to a host and its close relatives in the family or order. If, however, there are two forms attacking species of the same family but showing clear morphological differences, then independent specific rank has been given to them.'

In this investigation, the concept of a species as accepted by Mhatre and Mundkur (1945) has been followed. Five new species of *Synchytrium* on seven new hosts are recorded.

The specimens of the species reported here are deposited in the Herbarium of the Botany Department, Agra College, Agra and the *Herb. Crypt. Ind. Orient.* of Indian Agricultural Research Institute. New Delhi.

SYNCHYTRIUM PHASEOLI-RADIATI Sinha & Gupta, sp. nov.

Gallae in foliis atque culmis, sparsae constantius, maxime singulares, raro compositae, globosae, cupulatae, diametro 0.4-0.6 mm. Hypnosporae singulares in cellis hostibus epidermatibus, globosae, leves. fusae subnigrae, magnitudine 165-200μ (aestimatio media 184μ) diametro cum episporio 13-16.5μ crasso.

In foliis atque culmis *Phaseoli radiati* Linn. sp. Agra, 22-8-1948 ; leg. S. Sinha & S. C. Gupta, typus ; *Phaseoli mungo* Linn. Agra, 12-9-1948 ; leg. S. C. Gupta & S. Sinha ; *Cajani cajan* Millsp. Agra, Sept. 1948, leg. S. C. Gupta & S. Sinha ; *Crotalariae junceae* Linn. Agra, Sept. 1948, leg. S. C. Gupta & S. Sinha.

Galls on leaves and stems, scattered rather uniformly, mostly single, rarely compound, spherical, cupulate 0.4-0.6 mm. in diameter. Resting sporangia solitary in epidermal host cells, spherical, smooth, dark brown, measuring 165-200μ (mean value 184μ) in diameter with an epispore 13-16.5μ thick.

On leaves and stems of *Phaseolus radiatus* Agra, 22-8-1948 leg. S. Sinha & S. C. Gupta, type ; *Phaseolus mungo* Agra. 12-9-1948 leg. S. C. Gupta & S. Sinha ; *Cajanus cajan* Agra, Sept. 1948 leg. S. C. Gupta & S. Sinha ; *Crotalaria juncea* Agra, Sept. 1948 leg. S. C. Gupta and S. Sinha. The diagnostic features of *Synchytrium on Phaseolus mungo* reported by Patel *et al* are very different and therefore a new species is suggested here.

SYNCHYTRIUM HIBISCI Gupta & Sinha, sp. nov.

Gallae in foliis atque culmis, sparsae singulariter vel coalecitae copiositer in contagionibus duris, gallae globosae 0.5-0.7 mm. magnitudine. Hypnosporae singulares in cellis hostibus epidermatibus, globosae, leves, magnitud. 182-210μ (aestimatio media 200μ) diametro cum episporio 20μ crasso.

In foliis atque culmis *Hibisci esculenti* Linn. Agra, 12-9-1948, leg. S. C. Gupta & S. Sinha typus

Galls on leaves and stems, scattered singly or coalescing abundantly in severe infections, solitary galls spherical, 0.5-0.7 mm. in diameter. Resting sporangia solitary in epidermal host cells, spherical, smooth. measuring 182-210μ (mean value 200μ) in diameter with an epispore 20μ thick.

On leaves and stems of *Hibistus esculentum* Linn. Agra, 12-9-1948. leg. S. C. Gupta & S. Sinha, type

Synchtrium trichosanthidis Mhatre & Mundkur in *Llyodia* **8**, p. 126, 1945

Host surface covered with minute, white galls measuring 0.15-0 3 mm. in diameter. Resting sporangia solitary in epidermal cells, smooth, spherical, dark brown measuring 70-80μ (75μ mean value) in diameter, with an epispore 5.8-8.2μ thick.

On leaves, stems, flowers and fruits of *Cucumis melo* var. *momordica* Agra, Sept. 1948, leg. S. C. Gupta & S. Sinha, and *Cucumis melo* Var. *utilissimus* Agra, August 1950, leg. S. C. Gupta & S. Sinha.

SYNCHYTRIUM MELONGENAE Gupta & Sinha, Sp. nov.

Gallae in foliis. minutae, copiosae secundum venas, repertae singulares vel coalescitae, maxime globosae, raro ovatae. Hypnosporae singulares in cellis hostibus epidermatibus, globosae, leves, magnitud. 56-76μ (aestimatio media 66μ) diametro cum episporio 8-10 μ crasso.

In foliis *Solani melongenae* Linn. Agra, 26-9-1948 leg. S. C. Gupta & S. Sinha typus.

Galls on leaves, minute, abundant along the veins, occurring singly or coalescing, usually spherical, rarely oval 0.2-0.3 mm. in diameter. Resting sporangia solitary in epidermal host cells, spherical, smooth, measuring 56-76μ (mean value 66μ) in diameter with an epispore 8-10μ thick.

On leaves of *Solanum melongena* Linn. Agra, 26-9-1948, leg. S. C. Gupta & S. Sinha, type

SYNCHYTRIUM SESAMI Sinha and Gupta, Sp. Nov.

Gallae in foliis atque culmis, praecipius in virgis inventibus laterlibus, non minutae, singulares vel coaliscitae facientes crustam coccineam in exemplis

siccis ; gallae in foliis 0.3-0.43 mm. diametro, in culmis 0.6-0.7 mm. Hypnosporae singulares in cellis hostibus epidermatibus, globosae leves, fulvae oliagineae magnitud. 172-201μ (aestimatio media 188μ) diametro et in foliis et culmis ; episporeo 10-13μ crasso.

In foliis atque culmis *Sesami indici* Linn. Agra, 12-9-48, leg, S. Sinha & S. C. Gupta, typus ; Meerut (U. P.), Sept. 1950, leg. M. R. Sharma

Galls on leaves and stems, more specially on young lateral shoots, not minute, single or coalescing forming a scarlet crust on dry specimens ; galls on leaves 0.3-0.43 mm. in diameter, on stems 0.6-0.7 mm. Resting sporangia solitary in epidermal cells, spherical, smooth, olive-brown, measuring 172-201μ (mean value 188μ) in diameter both on leaves and stems ; epispore 10-13μ thick.

On leaves and stems of *Sesamum indicum* Agra, 12-9-1948, leg. S. Sinha and S. C. Gupta type ; Meerut (U. P.), Sept. 1950, leg. M. R. Sharma. Lacy's (1950) description of the *Synchtrium* on *Sesamum indicum* is entirely different from the diaghostic features of the fungus described here. A new species of *Synchytrium* on the same host is therefore suggested.

SYNCHYTRIUM VERNONIAE Gupta and Sinha, Sp. Nov.

Gallae in culmis, sparsae solitares, raro compositae, globosae, magnitud. 0.35-0.6 mm. diametro. Hypnosporae singulares in callis hostibus epidermatibus, globosae vel ovatae, leves, fusae, subnigrae ; sporangia globosa 106-149μ (aestimatio media 122 μ) diametro, sporangia ovata 89-99 $\times$ 13·2μ magnitudine, episporeo 1-10μ crasso.

In culmis *Vernoniae patulae* (Dryand) Merr. Agra, Sept. 1948 leg. S. C. Gupta & S. Sinha, typus.

Galls on stems, scattered, singly, rarely compound, spherical, measuring 0.35-0.6 mm. in diameter. Resting sporangia solitary in epidermal host cells, spherical or oval, smooth, dark brown ; spherical sporangia 106-149μ (mean value 122μ) in diameter, oval sporangia 89-99$\times$132 μ in size, epispore 8-10μ thick.

On stems of *Vernonia patula* (Dryand) Merr. Agra, Sept. 1948, leg S. C. Gupta & S. Sinha, type

SUMMARY

Synchytria collected in the district of Agra (India) during the years 1948, 1949 and 1950 have yielded five new species, on seven hosts, which are described and illustrated.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. B. B. Mundkur for his valuable suggestions and to Miss M. E. Gibbs, St. John's College, Agra for translating the diagnoses into Latin.

DEPARTMENT OF BOTANY
AGRA COLLEGE, AGRA

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EXPLANATION OF PLATE

Fig. 1. *Synchytrium trichosanthidis* on *Cucumis melo* var. *momordica*, showing infection of leaves, stems, flowers and fruits.

Fig. 2. *Synchytrium trichosanthidis* on *Cucumis melo* var *utilissimus*, showing infection on leaves, stems flowers and fruits.

Fig. 3. *Synchytrium hibisci* on *Hibiscus esculentum*, showing infection on stem.

Fig. 4. *Synchytrium sesami* on *Sesamum indicum*, showing infection on leaves, stems, and young lateral shoots.

Fig. 5. *Synchytrium vernonie* on *Vernonia patula*, showing infection on stem.


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LINKAGE RELATIONS IN SACCHAROMYCES OF GENES CONTROLLING THE FERMENTATION OF CARBOHYDRATES AND THE SYNTHESIS OF VITAMINS, AMINO ACIDS AND NUCLEIC ACID COMPONENTS.*

By

CARL C. LINDEGREN and GERTRUDE LINDEGREN

Southern Illinois University, Carbondale, Illinois, U. S. A.

(Accepted for publication May 2, 1951)

The regular Mendelian inheritance of the characters differentiating our selected breeding stocks of Saccharomyces has made it possible to locate the genes controlling the characters on the different chromosomes. Tetrad analysis is feasible in Saccharomyces and affords a unique opportunity for the detection of non-Mendelian segregations which would otherwise escape observation. When all the chromosomes are adequately mapped, a crucial test of the significance of the exceptional non-Mendelian tetrads will be possible.

THE ORIGIN OF MUTANTS AND THEIR INCORPORATION INTO THE BREEDING STOCK

The large variety of freely interbreeding species of Saccharomyces are differentiated by genes controlling the fermentation or hydrolysis of various carbohydrates, and the synthesis of some B-vitamins. The pedigree in which these genes were combined into our breeding stock has been described in detail (Lindegren, 1949). Ultraviolet treatment was also exploited as a source of mutants. X-rays produce translocations in addition to mutations and since translocations cause a high degree of sterility, ultraviolet treatment was the preferred means of inducing mutations. Ultraviolet radiation produced defects in genes controlling the synthesis of various amino acids, purines, and pyrimidines. Our breeding stock, like standard Saccharomyces, is capable of synthesizing these substances from ammonium.

The following procedure was used in the ultraviolet treatment of haploid cultures : haplophase cells are streaked on a full nutrient agar plate and treated with an Hanovia Ultraviolet Lamp SC 2537 for 2 minutes at 60 cm. The plate is then immediately subjected to sunlight for 15 minutes (Kelner, 1949). After 48 hours incubation, small colonies are picked from the plate and streaked on nutrient agar in test tubes. A loopful of cells from the growth on the nutrient agar slant is suspended in sterile distilled water to dilute whatever growth substances may be present.

*This work has been supported by grants from Anheuser-Busch, Inc. and by the National Cancer Institute, of the National Institutes of Health, Public Health Service.

A loop of this suspension (approximately 300 cells) is used to inoculate a tube of 10 cc. of liquid medium of the following composition :

Component	*Amount per liter*
asparagine	2.0 grams
KH_2PO_4	1.5 ,,
dextrose, anhydrous	20.0 ,,
$(NH_4)_2SO_4$	2.0 ,,
Mg $SO_4.7H_2O$	0.5 ,,
Ca $Cl_2.2H_2O$	0.33 ,,
KI	0.1 milligrams
Fe SO_4. $7H_2O$	0.25 ,,
Mn $SO_4.4H_2O$	0.04 ,,
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.02 ,,
H_3BO_3	0.60 ,,
Cu $SO_4.5H_2O$	0.04 ,,
Zn $SO_4.7H_2O$	0.31 ,,
thiamine hydrochloride	0.20 ,,
inositol	10.0 ,,
Ca pantothenate	0.20 ,,
pyridoxine hydrochloride	0.20 ,,
paraminobenzoic acid	0.05 ,,
nicotinic acid	0.20 ,,
biotin	2.0 micrograms

If no growth occurs in this tube after five days, it is assumed that a mutation has been induced. A screening procedure suggested by Dr. K. C. Atwood (personal communication) is used to detect a specific deficiency. The basic medium of the above composition is used and the liquid medium is supplemented in separate portions in 7 different ways (table 1). Supplement I contains lysine, arginine, histidine, aspartic acid, glutamic acid, choline and xanthine. It is the only supplement containing lysine. A culture which grows only in I and fails to grow in any of the others, is incapable of synthesizing lysine. Supplement II contains arginine, cystine, methionine, leucine, isoleucine, valine, and thymine. It is the only solution containing cystine, Supplements I and II contain arginine which is not present in any of the other supplements ; therefore, a yeast which grows both in I and II and fails to grow in any of the others requires arginine.

By this procedure, fertile mutant haplophase cultures have been obtained capable of interbreeding with our genetically marked stocks. Each mutant (with few exceptions) was detected in a single individual culture. Each mutant culture was mated to a normal strain and the hybrid was analyzed genetically for regular segregation. An elaborate series of matings was required to combine all the deficiencies into one breeding stock. One hybrid described below was heterozygous for 13 alleles, but not all of the gene differences could be classified in the progeny (table 2, Experiment 1).

CRITERIA FOR ESTABLISHING GENIC DIFFERENCES

The fact that two cultures arising from a tetrad can be differentiated into two contrasting categories suggests that the basis for their differentiation is the segregation of a single gene-pair. This suggestion is confirmed if the two haplophase parents were different and all the haplophase offspring are similarly differentiable into the same contrasting categories. Thus, if one parent be white and the other red, and each hybrid ascus produces two red and two white cultures, it is obvious that a single gene differentiates between redness and whiteness. With qualitative characters, the conclusions are clearcut. Quantitative characters present additional difficulties. For example, cells of the white cultures (adenine independent) planted into liquid media lacking adenine, produce turbidities within 48 hours, rated arbitrarily from 300 to 350 on a nephelometer, while the red cells (adenine dependent) inoculated into similar media produce turbidities varying from 0 to 50 in 6 days. We conclude that the white cells do not require adenine, while the red cells require it, growing slowly or not at all in its absence. The red cells grow equally well if supplied hypoxanthine instead of adenine. They also grow on adenylic acid, less well on inosine and even less on adenosine. The hereditary defect is, therefore, inferred to be the inability to transform some unknown substance into hypoxanthine and/or adenine.

When the quantitative differences are as widely separated and the exceptions as rare as in the case of adenine dependence, the differentiation is unequivocal. Whenever any precisely measurable and constantly recurring difference can be demonstrated (even if the deficiency be not absolute) a single gene difference can be inferred. Since syntheses occur in cycles. the substance supplied in the medium may be only remotely related to the substance whose synthesis or deficiency differentiates the two genotypes.

This may be especially true when the difference between the amounts of growth in the deficient and complete media are small, although the constant difference may prove that a single gene controls the difference.

The differentiation of the adenine-, anthranilic acid-, histidine-, and uracil—dependent stocks was sharp ; growth in the deficient medium does not occur before at least 2 weeks has elapsed. The dependent lysine culture achieves growth in the deficient medium after 5 to 10 days and is therefore much more difficult to differentiate, since the interval between the slowest "independent" (3 days) and the fastest "dependent" (5 days) is so small. The inositol—and pyridoxine—dependent cultures gave irregular results and the data were not used, although the parents were clearly differentiable with regard to these deficiencies. A clear-cut differentiation regularly segregating in the progeny of a hybrid justifies the conclusion that genes control the differences, but the differentiation is nearly always relative ; unless the time interval between the production of a heavy turbidity by the wild type culture and the beginning of growth by the dependent mutant is reasonably long, differentiation of "dependent" and "independent" clones is difficult. Precisely what occurs when a dependent culture acheives growth in deficient medium is not clearly understood ; in some cases, as in pantothenate (Raut, 1950), it has been shown to be mutation of a suppressor gene at a different locus.

LINKAGE CALCULATIONS

Table 2 shows results of hybridizing cultures heterozygous for many different alleles. The asci were classified as to the segregation for all possible combinations of two gene-pairs (A/a and B/b). Three types of asci are expected from an AB x ab mating : I, AB AB ab ab ; II, Ab Ab aB aB ; III, AB ab Ab aB. When the ratio of the three types is I : II: III= 1: 1: 4, the two genes are not linked and one or, both, are for from their respective centromeres. If I equal II, and if the ratio $\frac{I + II}{I + II + III}$ exceed 0.333, both genes are relatively closely linked to their respective centromeres. The ratio was calculated for all possible combinations of two gene-pairs and those combinations which showed significant deviations from 0.333 are listed in table 2. The procedure by which these data are interpreted has been described (Lindegren, 1949) and the results appear in table 3. It is clear that UR is nearer to its centromere than the genes G and α are to theirs. The distance for G and α differ from those calculated previously but the data are fewer and some variation is expected. The centromere-to-gene distance established by the most abundant data is that from the centromere to G. Data from several hundred asci indicate that this distance is 8. The present data gave a value of 15.5 for centromere to G and 9.5 for centromere to UR. If we assume that the distance 8 for galactose is approximately correct, we may calculate that UR is approximately 5 units from its centromere, $\left(\frac{9.5 \times 8}{51.5} = 4.9\right)$

THE INHERITANCE OF RED AND PINK COLOR

The ad 1 gene located on the first chromosome was derived from a culture obtained from Reaume and Tatum (1949). It was a pink culture which arose as a nitrogen mustard mutant. One of the offspring, a pink culture (ad 1) was treated with ultraviolet radiation and one of the mutants recovered was a deep red. This culture was mated with a normal white and 5 asci were analyzed. The results are indicated in table 4. The symbols 'a' for pink (ad 1) and 'b' for red (ad 2) are used in Table 4. Symbols 'A' and 'B', respectively, designate the normal alleles. These results indicate that the dark red was a double mutant. Both red cultures recovered from a white, white, red, red, ascus should be double mutants and 10 asci dissected from bybrid of a mating of one of these double mutant reds (a b) by a normal white gave the expected result (figures marked by asterisk in Table 4). Two matings of a red by a pink (from a red, red, pink, pink ascus) gave the expected ratio ; this mating is equivalent to a red (pink) by normal white. Pinks and reds recovered from red, red, pink, pink asci should be single mutants, respectively, and mating of these by normal white gave the expected results. Pink by pink produced only pink offspring and red by red produced only red offspring.

LINKAGE RELATIONS

The genes, ad 1 and ad 2, are independently assorted and red (ad 2) is epistatic to pink (ad 1). The ratio I : II : III = 4 : 3 : 19 shows that the genes are not linked and that ad 2 is relatively far from its centromere. The

white cultures are adenine-independent, while all colored cultures are adenine-dependent. The gene ad 2 is in the second chromosome. Cultures carrying it resemble cultures carrying ad 1 in their reversion to white except possibly for their behavior in the absence of methionine (Lindegren, 1949, Chap. 15). A considerable amount of data had established a significant distance of 46 units between galactose and melibiose. Table 2 (Experiment 1) shows ad 2 linked to both galactose and melibiose ; although the linkage is weak, the ratio of I: II in each mating strongly supports the validity of this conclusion. This places ad 2 in the second chromosome between G and ME.

The distance between the genes AN and HI is established as 25 morgans by the four matings shown in Table 2. The first matings were AN HI an hi, while the later matings were AN hi $\times$ an HI. The reversal of the genes in the parents of the hybrid gave the expected results.

Our previous experiments have established the separate existence of the 4 chromosomes carrying ADI, G, α and PB. The present data establish the separate existence of the four chromosomes : the first chromosome, and those carrying G, UR, and α. No data are available to indicate whether UR and PB are on the same or different chromosomes. The genetical data have therefore established a minimum of 4 chromosomes. LY (lysine), MA (maltose), SU (sucrose), MG (alpha methyl glucoside) have not as yet been linked to any other loci. MT (methionine), LE (leucine), AR (arginine), and PR(proline) have been tested and found to be regularly segregating, but hybrids containing these have not been analysed in quantity.

CYCLIC SYNTHESES

The synthetic cycles in yeast are approximately the same as in Neurospora (Tatum, 1949) and higher organisms. This is indicated by the fact that the indole deficiency may be satisfied by either anthranilic acid or tryptophane. The uracilless mutant is satisfied with uridine and less well with uridylic acid, but was satisfied neither by cytidylic acid nor cytidine (Loring and Pierce, 1944). Two lysine deficient cultures were isolated and neither is satisfied by alpha aminoadipic acid. The arginine deficiency was satisfied very slowly by ornithine and citrulline. The histidineless mutant is satisfied by the L—form, one half as well by the DL—form and not at all by the D—form.

SUMMARY

Selected inbred stocks of Saccharomyces produce hybrids in which Mendelian segregation occurs with a high degree of regularity. An occasional tetrad undergoes an irregular segregation which is detected by tetrad analysis. Irregular segregations occurring in maize or Drosophila with the same frequency would be undetected.

An effective method of screening yeast mutants for nutrilite deficiencies has yielded a group of fertile cultures which have been incorporated into the breeding stock.

Four (or five) chromosomes of Saccharomyces have been mapped for genes controlling the fermentation of carbohydrates and the synthesis of various nutrilites.

Chromosome I, PN (pantothenate), centromere, ADI (adenine), IN (inositol), PY (pyridoxine), and TH (thiamine). Total length 112 morgans.

Chromosome II, centromere, G (galactose), AD2 (adenine), ME (melibiose). Total length, 93 morgans.

Chromosome III, centromere, *a* (mating type). Total length 22 morgans.

Chromosome IV, centromere, PB (paraminobenzoic acid). Total length 22 morgans.

Chromosome V, centromere, UR (uracil). Total length 5 morgans.

Chromosomes IV and V may or may not be different; UR and PB have not been used in the same hybrid.

HI (histidine) and AN (anthranilic acid) are linked to each other (25 morgans) but have not yet been located on a chromosome.

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TABLE 1. *Supplements to Basic Medium Used for Screening Yeast Cultures for Nutritional Deficiencies**

I	II	III	IV	V	VI	VII
1.0 lysine	1.0 arginine	1.0 histidine	1.0 aspartic acid	1.0 glutamic	0.1 choline	0.5 xanthine
	1.0 cystine	1.0 methionine	1.0 leucine	1.0 isoleucine	1.0 valine	0.5 thymine
		1.0 glycine	1.0 alpha-alanine	1.0 serine	1.0 threonine	0.5 guanine
			1.0 tyrosine	1.0 phenylalanine	0.5 uracil	1.0 tryptophane
				0.1 folic acid	1.0 proline	0.5 hypoxanthine
					0.1 riboflavin	0.1 glutathione
						0.1 nicotinic acid

*The number in each block indicates the milligrams per liter

TABLE 2

*Tetrad Analyses and Linkage Calculation**

Experiment No. 1. 71 asci : in 50, all 4 spores survived ; in 20, only 3 ; in 1, only 2 survived.

10593*a*	AD2	AN	g	HI	IN	ly	MA	ME	mg	py	su	ur
					x							
10365*a*	ad2	an	G	hi	in	LY	ma	me	MG	PY	SU	UR

Mating				I	:	II	:	III	I+II / I+II+III	Linkage
a	G x	*a*	g	12		15		30	47.4	—
a	UR x	a	ur	14		16		28	51.7	—
G	ad2 x	g	AD2	20		8		32	—	40.0
AD2	ME x	ad2	me	15		9		36	—	45.0
AN	HI x	an	hi	31		2		26	—	25.4
G	me x	g	ME	10		9		42	—	49.0
G	UR x	g	ur	17		19		25	59.0	—

Experiment No. 2. 23 asci ; in 12, all 4 spores survived ; in 9, only 3 ; in 2, only 2 survived.

11015	AN	HI	ly	PY	UR
		x			
11040	an	hi	LY	py	ur

Mating					I	:	II	:	III	Linkage
AN	HI	x	an	hi	13		0		8	19.1
AN	PY	x	an	py	5		1		15	40.5
HI	PY	x	hi	py	3		1		17	50.0

Experiment No. 3. 9 asci

11433	an	HI	PY	
	x			
11461	AN	hi	py	

Mating					I	:	II	:	III	Linkage
AN	hi	x	an	HI	0		4		5	27.9
AN	py	x	an	PY	2		0		7	61.2
HI	PY	x	hi	py	1		0		8	44.5

Experiment No. 4.

54 asci ; in 29, all 4 spores survived ; 15, 3 spores survived ; in 2, 2 spores survived ; in 8, no spores survived.

11461 AN hi LY UR
x
11462 an HI ly ur

Mating	I	:	II	:	III	Linkage
AN hi x an HI	0		20		24	27.3

Experiment No. 5. 12 asci

11189 *a* an g MA me pn py th UR
x
11407 a AN G ma ME PN PY TH ur

Mating	I	:	II	:	III	Linkage
AN PN x an pn	6		0		5	23
AN PY x an py	2		0		9	41
AN TH x an th	1		0		10	45
AN ur x an UR	2		2		7	50
PN PY x pn py	1		0		11	46
PN TH x pn th	4		0		7	32
PN ur x pn UR	2		2		7	50
PY TH x py th	7		0		4	18
PY ur x py UR	3		2		6	45
TH ur x th UR	1		0		10	50

*Abbreviations as follows :

AN anthranilic acid
AD1 adenine 1 pink (on first chromosome)
AD2 adenine 2 red (on second chromosome)
G galactose
HI histidine
IN inositol
LY lysine
MA maltose
ME melibiose
MG alpha methyl glucoside
PB paraminobenzoic acid
PN Pantothenate
PY pyridoxine
SU sucrose
TH thiamine
UR uracil

TABLE 3

Calculation of distances from the centromeres

Ratio $\frac{I+II}{I+II+III}$	*Genes*	*Distance from graph** UR/ur	G/g	*a*/a
·590	G UR	9.5	15.3	
·517	*a* UR	9.5		20.3
·474	*a* G		15.3	20.3

*Lindegren (1949).

TABLE 4. *Tetrad Analysis of Red and Pink Hybrids*

MATING		ASCI ANALYZED									
		Phenotype				Genotype				Frequencies	
Phenotype	Genotype										
white × red (pink) normal × ultra violet mutant	AB × ab	white	white	red	red	AB	AB	ab	ab	1	1*
		red	red	pink	pink	Ab	Ab	aB	aB	1	2*
		white	red	pink	red	AB	ab	aB	Ab	3	7*
red × pink red from red, red, pink, pink ascus	Ab × aB	white	white	red	red	AB	AB	ab	ab	2	
		red	red	pink	pink	Ab	Ab	aB	aB	0	
		white	red	pink	red	AB	ab	aB	Ab	9	
red × white	Ab × AB	red	red	white	white	Ab	Ab	AB	AB	26	
pink × white	aB × AB	pink	pink	white	white	aB	aB	AB	AB	5	
pink × pink	aB × aB	pink	pink	pink	pink	aB	aB	aB	aB	8	
red × red (pink)	ab × Ab	red	red	red	red	ab	ab	Ab	Ab	4	

*Second Mating

THE ESTABLISHMENT AND MAINTENANCE OF PURE CULTURES OF RUST FUNGI

J. G. MANNERS

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Rust fungi are obligate parasites, which have not been grown in culture (Dickinson, 1949) , and hence cultures of rusts must be maintained on living plants. The techniques employed for the cultivation of rusts, therefore, differ in many respects from those employed in the case of fungi which can be grown saprophytically.

The steps involved in the establishment of a pure culture of a particular physiologic race of a rust from a field collection are as follows. A few rusted leaves are collected, together with particulars of host variety, locality, etc., and sent to the laboratory. Samples sent through the post in temperate countries arrive in good condition if despatched promptly. In the case of Yellow Rust, samples sent to me during my investigations on the physiologic specialization of that species (Manners, 1950), loss of viability in transit was usually due to delay, very hot weather, or extremes of humidity. A temperature of 25°C. kills spores of *Puccinia glumarum* in a few hours. but *P. graminis* and the Brown Rusts are more resistant to high temperatures and may be sent over long distances without loss of viability if suitably packed. Small samples sent loose in envelopes often dry up, and those sent very wet are sometimes mouldy and useless on arrival. In the writer's experience, material travels best when packed in a tin box with a few healthy leaves. Living plants growing in test tubes and inoculated with rust have also been used as a means of transport (D'Oliviera, 1939).

If many collections arrive simultaneously, or if the weather is unsuitable, it is not always possible to establish a culture at once. Rust spores keep best at 0-5°C. and 40% R. H. (Gassner and Straib, 1932 ; Mains and Jackson, 1926 ; D'Oliviera, 1939 ; Stakman, Levine and Loegering, 1943). The usual procedure is to place a few leaves from each collection in paper packets in a desiccator over 50% sulphuric acid (40% R. H.) inside a refrigerator. Spores stored in this way may be relied upon to retain their viability for atleast 3 months in the case of *P. glumarum* and for 6 months in the case of the other cereal rusts, and much longer periods have been recorded.

Plants for inoculation must be grown in a rust-free greenhouse and kept free of insects. The plants are inoculated, for stock cultures, on the second leaf, about 12 days after sowing, when the second leaf is as long as the first. Rust material from the field or from the refrigerator should be placed on damp filter paper in a Petri dish for a few hours before inoculation, to induce sporulation. Spores are transferred by means of a sterile paint-brush or scalpel (I prefer the latter); the leaf is drawn between the moistened finger and thumb before inoculation to remove the waxy cuticle. Inoculations may also be carried out hypodermically (Newton and Brown, 1934). The hands must be washed with soap and

water or spirit before each inoculation, and plants should be inoculated in still air, in a room where no rust cultures are maintained. Large batches of plant can be inoculated by spraying them with a spore suspension in 0·1% agar, (Gassner and Straib, 1931), contained in an automizer, but a scalpel is preferable for critical work. After inoculation the plants are placed under bell-jars or metal incubation chambers for 48 hours, to maintain the saturated atmosphere necessary for spore germination, and then transferred to the greenhouse bench. The bell-jars must be shaded and cooled with a water spray or similar device (Manners, 1950) when the greenhouse temperature exceeds 15°C in the case of *P. glumarum* or 20-25°C. in the case of other cereal rusts, otherwise the bell-jars become badly overheated and the spores fail to infect. After removal from the bell-jars, the plants are covered with spore-proof "Cellophane" cases, which exclude insects. These cases have the advantage that, unlike the lamp chimneys formerly employed, they are permeable to water. They must, however, be inspected regularly and replaced if they become torn or contaminated with growths of *Cladosporium.*

A variety susceptible to all known physiologic races must be used to establish a cultute of an unknown rust collection. For example, Norka wheat is susceptible to all wheat races of Yellow Rust, and being very resistant to mildew (*Erysiphe graminis*) eliminates any which may be present. Brown rust present in collections of Yellow Rust from wheat can be screened out by inoculation on Fong Tien barley, susceptible to all wheat rates of *P. glumarum,* but immune to *P. triticina. P. triticina* is usually maintained on Wilhelmina (Roberts, 1936), or Little Club (Chester, 1946) and *P. graminis* on Little Club.

Rust cultures can only be maintained in a greenhouse where suitable environmental conditions can be produced. In particular, a suitable temperature must be maintained. *P. glumarum* cannot be grown if the average temperature exceeds 20°C. the optimum being 13-16°C. The other cereal rusts have optima at 18-25°C. They are less sensitive to high temperatures than *P. glumarum* and can be grown at temperatures of up to about 30°C. At temperatures below 10°C in the case of *P. glumarum* or 15°C in the case of the other species, rust development is greatly retarded. Hence greenhouse heating, preferably thermostatically controlled, is normally required in the winter months in temperate countries. In summer, cooling is not usually necessary in temperate countries, if the greenhouse is suitably shaded and ventilated, except in the case of *P. glumarum.* In tropical countries cooling devices such as those described by Mehta (1940) are required. In winter it is difficult to grow cultures of most cereal rusts satisfactorily in Great Britain unless artificial lighting, preferably in the form of fluorescent tubes, is employed to supplement daylight. Unless a temperature and light controlled greenhouse is available, it is necessary to tide over unfavourable periods by storing material in the refrigerator.

In order to make sure that the races isolated are not mixtures, a single spore culture must be established from each collection, though in practice most collections contain only a single race. Single pustule cultures are easier to establish, but unsatisfactory, as it has been shown (Pieschel, 1931) that a single pustule may contain spores of more than one type. Most workers use the dry needle method of single spore

isolation introduced by Hanna (1924). The spores are scattered on a sterile slide and a single spore is picked up under the microscope on the tip of a dry sterile steel needle. This spore is transferred to a drop of water between two Indian Ink marks on the leaf and the plant is then incubated as usual. Success is only obtained under the optimal condition for the species concerned, when 5-10% of the spores give rise to infections. Other methods have been advocated by some investigators. *e.g.*, the spores may be picked up with a capillary pipette (D'Oliviera, 1939) or on a block of agar (Pieschel, 1931).

Tests on the differential hosts, to determine to what physiologic race a collection belongs, can only be carried out when the standard conditions for such tests are obtainable. This means that, unless temperature and light controlled greenhouses are available, the testing of cultures on the differential hosts is restricted to particular times of year. In the case of *P. glumarum*, the required combination of adequate light intensity and an average temperature of 15°C is usually only obtainable in Great Britain in March, April, May, September and October. The other cereal rusts may be tested at any time during the summer, since a temperature of 18-25°C is required, but tests cannot be made on them during the winter owing to poor light intensity (Brooks, 1944). In India, tests must be made in the cool season in a cooled greenhouse (Mehta, 1940).

Each new collection should be tested on the differential hosts as soon as possible, and all stock cultures at least twice a year. Inoculation and incubation on the differential hosts are carried out as for stock inoculations, but the first leaf is inoculated. Two cultures, if at all similar, must be tested side by side, in order to ensure identical environmental condition, before establishing them as two separate races, and there must be a consistent and clear difference in reaction type on at least one differential host (Stakman *et al.*, 1944). A new race should not be established unless its identity has been confirmed in several experiments. The seed of the differential hosts must be obtained from the original investigators on the rust species concerned, and must be kept genetically pure by rogueing and bagging the ears.

Rust cultures usually remain very constant over a period of years, though occasional mutations occur (*e.g.* D'Oliviera 1939). Abnormal reactions which are occasionally encountered are almost always due to environmental causes.

DEPARTMENT OF BOTANY
UNIVERSITY COLLEGE
SOUTHAMPTON, ENGLAND

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STUDIES IN THE BUNT OF RICE (*ORYZA SATIVA* L.)

By

S. CHOWDHURY

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INTRODUCTION

Bunt of rice caused by *Neovossia horrida* (Tak.) Padwick and Azmatulla Khan is a common and widespread disease of rice in Assam. It occurs in all the rice-growing areas of the State and infects all groups of rice, *ahu* (summer rice), *sail* or *sali*, *asra* and *aman* (winter rice) and *boro* (spring rice). In *ahu* and *boro* groups the disease is rarely noticed and appears to be less prevalent ; in the other groups of rice the disease is more common and observed more frequently.

Bunt of rice is known to occur in almost all the rice-growing countries of the world. Takahashi (1896) first reported the occurrence of this disease from Japan in 1896. Since then its occurrence has been reported from the United States of America by Anderson (1899) and Fulton (1908), India by Butler (1913), Indo-China by Duport (1912) and Vincens (1921), Burma by Rhind (1926), British Guiana by Stevenson (1926), China by Teng (1931) and Wei (1934), Philippines by Reyes (1933) and the Atlantic Islands and the Portuguese colonies by DaCamara and DaLuz (1938).

ECONOMIC IMPORTANCE

Estimates made by the different workers about the loss this disease causes differ considerably. Takahashi (1896) reported that this disease affects only a few grains. Fulton (1908), on the other hand reported that in South Carolina the disease causes a damage of as much as 25 per cent. Cook (1913) considered that it is probably the most serious of the diseases of rice of lesser importance. Butler (1918) stated that the amount of damage caused by this disease is usually slight and few individual grains in an ear, often not more than 2 to 3 being affected. Vincens (1921) and Rhind (1926) both state that the disease was unimportant. However, in one year Su (1933) in Mandalay found a loss of 2 to 5 per cent of the grains.

During the last six years the author carried out an exhaustive survey and collected random samples from the different rice-growing tracts of the State to assess the loss which this disease causes. As a result of this survey it has been found that there is considerable variation in the percentage of plants affected in the fields and in the number of grains that are bunted in an ear. These vary from field to field, from locality to locality and from year to year in the different groups of paddy. From all practical points of view it may be said that the disease is non-existent in the *ahu* and *boro* groups of paddy and therefore causes practically no

damage. Rarely 0.05 to 0.5 per cent of the plants are affected and not more than one to three grains in an ear are bunted. In the *aman* and *asra* groups of paddy the disease is more frequently noticed than in *ahu* and *boro*. Under usual conditions 0.05 to 1.5 per cent of the plants are attacked and 2 to 4 grains in an ear bunted. In *sali* paddy the disease, however, appears to be most conspicuous; in certain years in restricted localities 10 to 52 per cent of the plants have been found affected and the number of bunted

Plate I. Symptoms of the Disease

grains in an ear has been found to be 1 to 9. But normally 0.05 to 17.5 per cent of the plants are found attacked by the disease and the number of grains affected in an ear has been found to vary from 2 to 5. Taking all the paddy groups as a whole, it may be safely stated that the disease is of minor importance and does slight damage.

SYMPTOMS

The grains only are attacked by the disease and not until the grains begin to ripen does the blackening due to bunt becomes manifest at close range. Diseased panicles are carried by the culms in the same manner as the uninfected ones, and usually not all culms in the same stool bear diseased heads. The disease is recognised generally by the presence on the surface of the grains of a black, powdery or sooty mass of spores sticking together and usually clustered towards the tips of the unaffected hull or on their hairy portions. Ordinarily the affected panicles show only a few blackened grains in one or two spikelets, but in fairly severe cases of infection more spikelets show bunted grains, but bear no fixed position in the ear. Sometimes the infected grains gape or show a little separation of the tips as a result of the slight enlargement of some of the kernels, and occasionally also a short, somewhat beak-shaped or inverted, spur-like outgrowth comes out by the rupturing of any portion of the keel of the larger glume or lemma in severe infection. This protruding spur-like structure is nothing but the shrunken or atrophied remnant of the eaten out endosperm, which remains in tact, extruded through the inclosing membrane and the inner glume by force through the multiplication of the spores inside. Some of the bunted grains do not show any external symptoms whatsoever and expert observers often find it difficult to detect these. Such grains when held up against strong light show a black mass inside and when split open show the characteristic black dust of spores of the fungus.

Internally on carefully splitting up the bunted grain it will be found that the kernel is covered with a more or less transparent or gray membrane with the fungus transforming the ovary partly or wholly into a dense sooty black mass of spores. In the advanced stages the kernel is wholly replaced by spores and this is what causes actual loss. But it is not rare to find that the kernel is only partially destroyed in which case the partially destroyed grain when sown sprouted. Plate I shows the symptoms of the disease.

EFFECT OF THE DISEASE ON THE HEIGHT AND TILLERING OF THE PLANT

Reyes (1933) reports that bunted plants become shorter in height and that they form lesser number of tillers than the normal healthy paddy plants. Camus (1935) has also reported the stunting of the affected plants. Quite a large number of data collected, however, failed to confirm this finding. The height of 400 healthy and 400 diseased plants of each of four varieties of rice was measured and the number of their tillers counted. The plants were selected at random from the same plots. The data are presented in Tables I and II and in Figs. 1-4.

TABLE I

Comparative data showing the height of healthy and bunted plants of rice

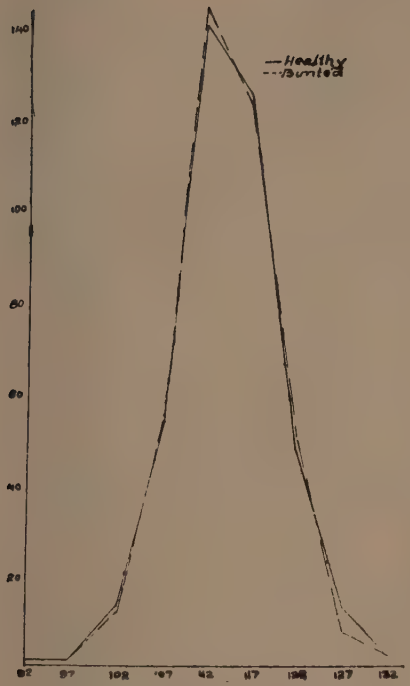
	Swarnasail		Latisail		Kerrsail		Nagrasail	
	H	B	H	B	H	B	H	B
Height (Cm.) Mean	119.01	118.12	114.38	114.36	111.75	112.24	118.88	118.49
S.D.	7.51	8.21	5.53	5.46	7.81	7.39	7.81	7.74
S.E. of Mean	0.375	0.410	0.277	0.273	0.391	0.370	0.391	0.387

TABLE II

Comparative data showing the number of tillers in healthy and bunted plants of rice

	Swarnasail		Latisail		Kerrsail		Nagrasail	
	H	B	H	B	H	B	H	B
Mean	8.93	8.97	9.28	9.28	8.83	8.61	10.17	9.1
S.D.	2.51	2.46	2.48	2.37	2.70	2.76	2.80	2.795
S.E. of Mean	0.126	0.123	0.124	0.119	0.135	0.138	0.140	0.138

H=Healthy ; B=Bunted.



1 Height of plants (Latisail) in cm.

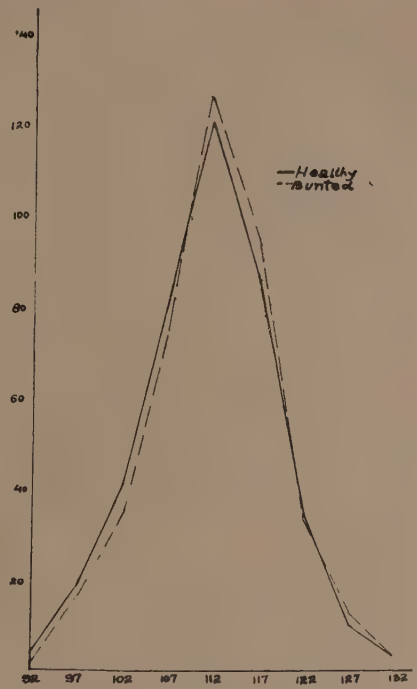


Fig. 2H.eight of Platus (Kerrsail) in cm.

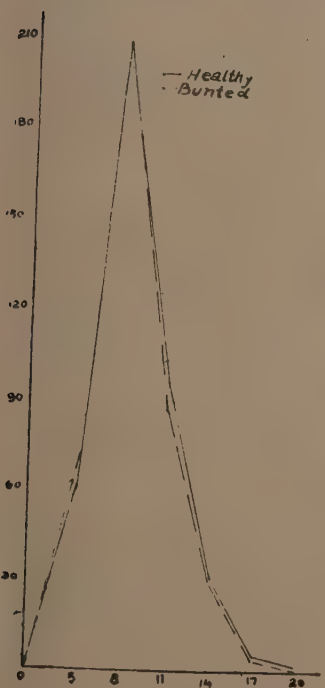


Fig. 3. Number of Tillers (Kerrsail)

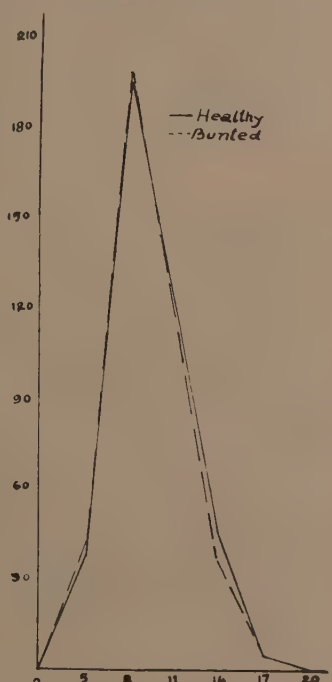


Fig. 4. Number of Tillers (Latisail)

It will appear from the data presented in Tables I and II and in Figs 1-4 that there is no significant difference in height and in number of tillers between bunted and healthy rice plants. In this connection it may be mentioned that Harwood (1892), Potter and Coons (1918), Sampson and Davis (1927), Rodenhiser (1931) and Mitra (1937) have reported on the retarding influence on the development of the wheat plant, significant reduction in the length of culms and height of the wheat plant due to attack by the various species of *Tilletia* whereas Selby (1898) and McAlpine (1910) have found no difference. It may, however, be stated that as this disease is not systemic it cannot have any effect on the height and tillering of the plant.

MORPHOLOGY OF SPORES

Spores in the various stages of development are present usually inside the ovary. These are variously compressed assuming different forms and sizes. The immature spores are hyaline, sub-hyaline or slightly yellowish, the latter two exhibiting blunt spines. The hyaline spores are generally, smooth. Some of the young spores are shrunken and generally elongated ; others are without contents or spinous wall-projections but with peripheral thickening.

The mature spores (Fig. 5) are opaque, olive-brown to black, globose to sub-globose or occasionally elliptical and the surface is provided with slightly coloured or hyaline spinous, scale-like projections. These echinulations which are more conspicuous in less opaque spores are frequently curved and pointed at the apex. More carefully examined, however, the 'spines' are found to be thickened blunt pegs formed in the substance of a thin hyaline membrane, which persists until after maturity. Seen in surface view, the blunt ends of the thickenings appear dark, the spaces between being light, so that a reticular effect of light bands is given. The hyaline membrane is somewhat gelatinous when moist and the adhesive properties of the spores are due to it. The mature spores measure from 18 to 26 μ in diameter.

FACTORS INFLUENCING DEVELOPMENT OF BUNT

Careful field observations carried out during the last six years show that the bunt is present every year but the intensity of bunt varies from year to year and from locality to locality. Even in the same variety of rice the intensity of attack has been found to vary from year to year. From this it appears that weather and other environmental factors probably play an important part in the development of the disease. Of course the presence of an abundance of viable spores of the fungus in the soil nearby is very important for the development of the disease. If proper and adequate inoculum is lacking, the disease will not develop even if the weather and other environmental factors are present.

Except Reyes (1933) and Camus (1935) no other investigators appear to have carried out any observations and studies in this respect. According to Reyes (1933) rains followed by hot sunshine immediately after sowing are conducive to the rapid development of the disease as the emerging seedlings are at once exposed to the possibility of infection. He further states

that possibly excessive damp soil or impermeable soil continuously under water during the entire growing period of the plant are also contributory agencies. Camus (1935) found the disease most serious in irrigated fields, the plants becoming predisposed during dry weather. The present investigator, however, has so far failed to confirm these findings. The field observations so far carried out by him, on the other hand, reveal that light and frequent rains during the latter part of October and early November when the paddy plants are in flowers, help the development of the disease. If the weather conditions are dry during this period the disease is appreciably less ; if, on the other hand, the weather conditions are moist and humid during this period and there are light and frequent spells of rain with short intervening dry periods the incidence of the disease is severe. This is probably due to the fact that damp and humid weather conditions with the frequent light showers at intervals during this period, on the one hand, help the germination of the bunt spores lying in the soil and plant debris and, on the other hand, afford conditions favourable for the infection of the rice flowers easily by the sporidia of the fungus. It is during this part of the year that the important groups of paddy (*aman*, *asra* and *sali*) are in flower and as the present investigator (Chowdhury, 1946) has already shown that the infection of this bunt takes place through flowers, sporidia must be carried to the flowers during this period of the year for the successful infection of the flowers by the parasite. In Assam the monsoon ceases by August and from September dew starts to fall and there are also occasional spells of showers. The temperature also varies from 25 to 30° C. which is favourable for the germination of the spores.

Under field conditions *aman* and *asra* have been found less affected than the *sali*. This is very probably due to the fact that *asra* and *aman* are grown in low-lying tracts in deep water. The spores which might remain in the soil go under a few feet of water; high land which can harbour the spores are rarely come across. Because of this only a very negligible percentage of the plants are affected only in areas where high land is nearabout and wherefrom there are possibilities of the sporidia of the fungus being carried by the wind. *Sali* paddy, on the other hand, is grown on high land. Only a few inches of water stand below the crops and the small plots in which it is grown are encircled by high land called *alis*. It is on these *alis* (strips of high land which form the boundary of the small plots of land) that the spores of the fungus can lie dormant. The spores germinate under favourable conditions and the sporidia they produce are carried by the wind which in turn infect the rice flowers. As the plants are very near, the chances of failure are less and consequently a higher percentage of infection is noticed in *sali* paddy.

In the case of *ahu* and *boro* paddy as previously stated the disease is practically non-existent and is extremely rarely noticed. Careful observations so far made failed to indicate that the development of the disease in these two groups of paddy is at all influenced by weather conditions. The factor which primarily influences the development of the disease in these two groups of paddy appears to be the presence of sufficient viable spores in the soil when these groups of paddy are in the fields. The *sali*, *aman* and *asra* groups of paddy which are more susceptible to bunt are harvested in the months of December and January. From these crops the bunt spores are shed in the soil during these months. But as the bunt spores do not

germinate before a rest period of at least four months the chance of infection of *boro* paddy by such spores is remote as *boro* flowers in March. The spores which might have been shed from an infected *boro* crop, on the other hand, had very probably lost their viability after remaining under water for 5 to 6 months as the areas where *boro* is usually grown goes under deep water during the monsoons and remains so for some months. The chance of infection of *ahu* paddy is also very less because although flowering in *ahu* is periodically fixed, it is grown usually during the months of March-May. Consequently, *ahu* flowers in May and its infection by sporidia from the germination of the bunt spores which might have been shed from the previous winter paddy is remote. During May a very negligible percentage of the spores is likely to germinate. Further, on account of the heavy monsoon rains the sporidia which are carried by the wind and deposited on the flowers are likely to be washed away by the heavy rains. It is very probably because of the existence of these conditions that the *ahu* paddy is very rarely affected by the disease.

Mode of Transmission of the Bunt

Until recently bunt of rice was considered to be a seed-borne disease. Anderson (1899) stated that he had found hyphae in the stem tissues of bunted plants and Butler (1913) had confirmed that finding but on what grounds these authors presumed that the mycelium was that of the bunt fungus is not clear. In the Philippines, Reyes (1933), as a result of field and pot experiments, came to the conclusion that rice bunt is systemic and transmitted through the spores adhering to the seeds. The present author (Chowdhury, 1946) as a result of his studies indicated for the first time that the disease is not seed-borne but that the infection takes place through the flowers. He has since then carried out further experiment to confirm his findings and the results are presented here.

There are three possible ways by which bunt can be perpetuated from year to year. Exhaustive experiments were, therefore, carried out in these three directions during the last four years and the results achieved are presented below.

(*i*) *Seed infection.* Seeds of variety *Latisail* were surface sterilized with 1 : 1000 mercuric chloride, washed several times with sterile water and treated as follows :

(*a*) Seeds were smeared thoroughly with the spores of the fungus ;

(*b*) Seeds were smeared thoroughly with the spores of the fungus which were previously soaked in water for 7 days ;

(*c*) Seeds were smeared with the sporidia after germinating the chlamydospores ;

(*d*) Seeds were given no treatment but sown as they were ; these served as contol.

Seeds were sown in seed beds at Karimganj on June 20, 1946. The seedlings were transplated in plots 100 square feet in area on August, 20

1946. There were 100 plants in each plot. Fifty ears in each plot were enclosed in paper bags before they had emerged out of the boot leaves. Incidence of the bunt was ascertained just before harvest when the grains had matured by careful examination of all the grains in the ears. The results are recorded in Table III.

TABLE III

Incidence of bunt in a crop raised from artificially infected seeds

Treatment	No. of ears which got infected	
	Ears exposed to natural infection	Ears Bagged
(*a*) Seeds smeared with spores	7	Nil
(*b*) Seeds smeared with presoaked spores	6	Nil
(*c*) Seeds smeared with sporidia	5	Nil
(*d*) Seeds without treatment	7	Nil

It will be evident from the data presented in Table III that infection occurs only in some of the ears which had not been bagged irrespective of whether the seeds were healthy or smeared with the spores of the parasite. When the ears were bagged infection did not take place indicating that the disease is not seed-borne. The experiments were repeated in 1947, 1949 and 1950 and similar results were obtained.

(*ii*) *Soil infection.* Small plots each 100 square feet in area were infected with the viable spores of the fungus on August 15, 1946. Similar plots but without infection of the soil with the spores of the fungus were kept as control. Two lots of seedlings of variety *Latisail* were raised in two plots of land ; one plot was infected with the viable spores of the fungus before sowing the seeds while the soil of the other plot was not infected with the fungus. Sowing in the seed bed was done on June 10, 1946. Transplanting was done on August 27, 1946. Seedling raised in the plots previously infected with the spores of the fungus were transplanted on to those plots where the soil was infected with the viable spores of the fungus. Seedling raised on plots the soil of which was not infected were transplated on the plots the soil of which was not infected with the spores of the fungus. When the flowering was about to start and the ears were still enclosed in the boot leaf 50 ears in each plot were enclosed in paper bags to exclude any chance of external infection. Just before harvest the ears were very carefully examined and the incidence of bunt noted. The results are recorded in Table IV.

TABLE IV

Incidence of bunt in soil infected plots.

Treatments	Artificially infected plots		Control plots.	
	Ears exposed to infection	Ears Bagged	Ears exposed to infection	Ears Bagged
Sowing date on seed bed June, 10. Transplanting date ; August, 27	10	Nil	12	Nil

An examination of the data recorded in Table IV indicates that bunt develops both in plots artificially infected with the spores of the fungus and in control plots. Its total absence in ears that had been enclosed in paper bags proves that it is not soil-borne and is not systemic. The experiments were repeated in 1947, 1949 and 1950 and exactly similar results were obtained.

(iii) *Floral infection.* A large number of tests were conducted to determine whether the disease could be transmitted through the flowers. Four varieties of paddy (*Latisail, Kerrsail, Nagrasail* and *Swarnasail*) were selected for the tests and when the ears were in the boot stage, they were bagged to prevent any possible aerial infection. A large number of chlamydospores were then germinated and sporidia were kept ready for infecting the flowers. When anthesis started, a large number of ears that had been bagged were infected with the sporidia using the vacuum method devised by Moore (1936) and again enclosed in bags. An equal number of bagged ears were kept as controls. The mature ears were harvested and very carefully examined for the presence of bunt. The results of these tests are recorded in Table V.

TABLE V

Results of floral infection of paddy with sporidia

Variety	Per cent. infection	
	Inoculated	Not Inoculated
Latisail	45	Nil
Kerrsail	89	Nil
Nagrasail	78	Nil
Swarnasail	52	Nil

From the data recorded in Table V it will be evident that infection of paddy flowers with the sporidia of the fungus at anthesis can bring about the disease. Typically bunted grains, some of which were completely affected and others partially, have been obtained. Ears that had been bagged but were not infected did not, however, show any bunt infection. These tests were repeated during the years 1947, 1949 and 1950 and exactly similar results were obtained.

From all these tests it can be finally and safely concluded that the bunt of rice is not a soil or seed-borne disease; the infection takes place invariably through the flowers. What happens in nature is that the spores of the fungus from the previous season lying about in the fields either in the soil or on paddy and other stubble germinate at the advent of favourable conditions producing a large number of sporidia. These sporidia carried by the wind, settle on the ears in the anthesis or dough stage and the grains ultimately get attacked.

Reyes (1933) planted lightly bunted grains in sterilized soil and compared the results with those from healthy grains similarly planted. A large proportion of the bunted grains gave rise to bunted plants, whereas the healthy grains gave entirely healthy plants. From this he concluded that the disease is seed-borne and systemic in nature. But this does not give adequate proof that the disease is systemic, for infection may have taken place through sporidia formed late in the growth of the plant and carried to the flowers. This is very probably what actually happened because no precaution was taken by Reyes to bag the flowers and to protect them from infection by sporidia of the fungus which might be carried by the wind. Reyes (1933) also attempted floral infection but obtained negative results. This is because of the fact that while carrying out the floral infection he used the chlamydospores. In nature the infection of the rice flowers is actually brought about by the sporidia and not by the chlamydospores; so had he carried out the infection tests with the sporidia he would have surely got positive results. When the infection is carried out by the chlamydospores the conditions of the environment and the flower must be such that these are favourable for the germination of the chlamydospores. If it is not so the chlamydospores cannot germinate and naturally the flowers cannot get infected by the fungus. The present investigator carried out infection tests both with sporidia and chlamydospores. He found that inoculation with sporidia gave consistently better results and higher percentage of infection. Inoculation with chlamydospores, on the other hand, gave very poor results and were almost always unsuccessful.

SUMMARY

Bunt of rice caused by *Neovossia horrida* is a common and widespread disease of rice in Assam. It attacks all groups of rice, *ahu*, *sali*, *asra*, *aman* and *boro*. The disease, however, is most prevalent on *sali* varieties.

Survey carried out during the last six years show that the disease causes negligible damage. A very low percentage of the plants are attacked and that only 2 to 4 grains in an ear are usually infected.

The symptoms of the disease have been described.

Exhaustive data collected show that there is no significant difference in height and in number of tillers between bunted and healthy rice plants.

Morphology of the spores has been described.

Field observations carried out indicate that the development of the disease is influenced by climatic conditions and that a larger percentage of the plants are affected when light and frequent rains occur during the later part of October and early November.

Experiments carried out over the last six years to determine the mode of transmission of the disease revealed that the infection takes place through flowers.

My thanks are due to Dr. B. B. Mundkur, Dy. Plant Protection Adviser, Ministry of Agriculture, New Delhi for going through the manuscript and valuable suggestion.

Plant Pathological Laboratory
Jorhat, ASSAM

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EFFECT OF INSOLATION AND CHEMICAL TREATMENT IN RELATION TO STORING OF JUTE SEEDS

By

T. Ghosh, M. Basak and B.C. Kundu

Jute Agricultural Research Institute, Hooghly
West Bengal

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INTRODUCTION

The seed-borne nature of the "stem-rot" disease of Jute, caused by *Macrophomina phaseoli* (Maubl.) Ashby was established by Varadarajan and Patel (1943), while working in this laboratory during 1940-43. They have given a description of the distinguishing characters of the diseased seed. It applies mainly however to seeds of *Corchorus capsularis*, which are usually of various shades of brown. Samples of seeds of *capsularis* jute (local name 'deshi', 'suti' or 'titapat') can be graded from deep 'sepia brown' to 'dull onion brown' depending on the differences in the localities where they were grown. In any given sample, the diseased seeds are paler than the healthy ones, although the healthy seeds may be paler, when compared to the healthy seeds of another lot of deeper brown type. The seeds of *Corchorus olitorius* (local name 'bogi', 'deo', 'mithapat') are 'dusky green' to 'lily green'. As in the case of *capsularis* seeds, the paler, shrunken and shrivelled seeds are usually non-viable and mostly diseased. "Stem-rot" and "root-rot" diseases may cause considerable loss of crop as well as deterioration in the quality of the fibre. Hence Varadarajan (1946) sought absolute control of the seed-borne fungus by treatment of seeds with organic mercuric compounds. The aim was to neutralize this important primary source and to bring the chances of blight and disease in general to the minimum.

The present work is a continuation of the same investigation and was undertaken with a view to developing methods of storage of seeds in good condition.

MATERIALS AND METHODS

The technique used was the same as described by Varadarajan *et al* (1946) ; seeds of D154 type of *C. capsularis* were used. The sampling method was statistically checked. In all cases the medium used was standard potato dextrose agar. All the tests were carried out at an uniform temperature of 33°C (± 0.5°C) in the incubator.

EXPERIMENTS, RESULTS AND DISCUSSIONS

Two organo-mercuric compounds, mentioned herein as compound I* and compound II**, have been used in all experiments. Previously the practice in this laboratory was to treat the seeds for two minutes, prior to sowing. To determine the optimum duration, necessary for treatment with compound I, the following routine work was executed. Three out of four lots from a diseased seed sample were treated with Compound I for 2, 5 and 10 minutes respectively. After treatment, the excess chemical was sieved out and the samples were stored in airtight glass jars. The fourth untreated lot was kept as control. Tests were carried out from time to time. Results are tabulated as follows :—

TABLE I

Result of treating Jute seed for different durations

Date of observation	Sample stored after—							
	Two minutes' treatment		Five minutes' treatment		Ten minutes' treatment		Control	
	(G.P.)	(D.P.)	(G.P.)	(D.P.)	(G.P.)	(D.P.)	(G.P.)	(G.P.)
24.10.44	76.6	2.9	68.5	6.0	74.7	0.0	76.1	8.9
4.11.44	78.2	6.8	73.8	3.6	64.2	0.8	70.2	29.9
14.11.44	82.6	1.4	82.1	1.8	68.5	0.0	70.2	8.7
12.12.44	76.6	0.0	78.6	0.0	72.3	0.0	81.4	17.4
23.12.44	76.4	0.0	80.4	0.0	74.3	0.0	84.2	11.6
2. 1.45	78.2	0.0	82.2	0.0	76.2	0.0	80.2	8.6
10. 1.45	80.4	0.0	82.2	0.0	80.2	0.0	78.4	5.1
25. 1.45	80.6	0.0	76.6	0.0	80.2	0.0	76.8	11.3
30. 1.45	85.8	0.0	82.6	0.0	82.1	0.0	72.1	10.4
16. 2.45	81.2	0.0	83.0	0.0	81.2	0.0	71.0	14.2

G.P. = Germination Percentage
D.P. = Disease percentage

*Compound I = Tolyl mercuric acetate, as Agrosan G containing 1% mercury.
**Compound II = Ethyl mercuric chloride as New Improved Granosan containing 2% mercury.

From the above table it is apparent that the fungistatic action of the dust is better utilized by increasing the duration up to 10 minutes. Germination percentage is not adversely affected, if duration is increased to 10 minutes. Readings taken in the last week of December onwards show complete absence of any fungal growth. It was, therefore, considered that post-treatment storing may prove successful and beneficial.

To elaborate this point, the second phase of investigation was started on October 26th, 1945. *Capsularis* (D154) seeds were grown at Dacca Farm under conditions favourable for pod-infection. Seeds were collected on 26th October, 1945 and different lots were dried in the sun for different lengths of time *viz.*, 0,1,2,3,4,5,6 and 7 days and were stored in air-tight glass jars.

A small part of each of the above eight samples was treated with compound I for 10 minutes and another part with compound II for 30 seconds and stored in air-tight glass jars.

The moisture contents of the following samples were recorded immediately before storing and are tabled as below —

TABLE II

Moisture content of seeds

Sample No.	Number of days for which the seeds were exposed in the sun			
	Immediately after harvest	1 day	3 days	4 days
1.	21.76	16.60	8.52	7.2
2.	19.86	15.90	8.56	7.6
3.	21.30	17.40	8.95	7.0
4.	22.78	17.14	9.40	7.9
5.	21.22	16.94	8.66	7.8
Mean	value 21.38	16.79	8.76	7.5

It can be pointed out here that at the harvest stage, the moisture content is as high as 21.38 per cent on an average. (This varies with the relative humidity and temperature of the atmosphere, prevailing at the time of seed collection). It is brought down to 7.5 per cent after exposure in the sun for 4 days.

During our routine analyses of farmers' samples of jute seeds from different jute growing districts, we found that the number of non-viable and diseased seeds was abnormally high in many cases. The cause of this, we assumed, was due either to (1) improper drying in the sun or (2)

collection of seeds at immature stage coupled with infection. That proper drying in the sun has intimate relation with good storage will be apparent from the following experiments.

The stored samples, as described above, were next tested. Percentage of germination and disease of all the samples were recorded from time to time. The control used was D154 seeds grown in 1945 in the same field of Dacca Farm.

TABLE III

(1st Test—October, 1945)

Germination and disease percentage of stored seeds dried in the Sun, Sun × compound I, Sun × compound II

Days of Sunning	Sunning only		Sunning × compound I		Sunning × compound II	
	(G.P.)	(D.P)	(G.P.)	(D.P.)	(G.P)	(D.P.)
0.	52.1	23.0	65.0	2.1	69.5	2.2
1.	69.2	24.3	62.4	1.4	81.0	3.9
2.	74.5	24.0	72.5	1.8	74.5	2.8
3.	74.8	19.0	78.5	1.3	81.4	3.2
Control	67.2	37.3	70.8	37.3	71.4	37.3
C.D.	6.3	8.2	6.3	8.2	6.3	8.2

G.P.=Germination percentage
D.P.=Disease percentage

TABLE IV

(2nd Test—February, 1946)

Germination and disease percentage of stored seeds dried in the Sun, Sun × compound I, Sun × componnd II

Days of Sunning	Sunning only		Sunning × compound I		Sunning × compound II	
	(G.P.)	(D.P.)	(G.P.)	(D.P.)	(G.P.)	(D.P.)
0.	68.2	24.6	69.2	3.6	66.0	2.2
1.	82.8	24.3	73.0	4.0	74.7	2.5
2.	81.9	24.3	79.3	2.6	83.3	2.5
3.	80.6	16.0	84.4	2.5	82.1	2.6
4.	76.6	11.5	77.8	2.9	82.0	2.2
5.	79.9	14.8	83.9	2.7	84.7	2.2
6.	76.8	11.0	81.9	2.8	76.0	2.6
7.	76.5	10.6	82.7	2.2	83.2	2.6
Conttol	40.9	18.9	60.8	19·2	59.6	23.2
C.D.	6.5	4.7	9.5	3.2	6.0	0.79

G.P.=Germination percentage
D.P.=Disease percentage

TABLE V

(3rd Test—March, 1946)

Germination and disease percentage of stored seeds dried in the Sun, Sun × compound I, Sun × compound II

Days of Sunning	Sunning only		Sunning × compound I		Sunning × compound II	
	(G.P.)	(D.P.)	(G.P.)	(D.P.)	(G.P.)	(D.P.)
0.	53.7	17.8	62.1	2.0	62.6	1.0
1.	85.8	15.6	74.1	1.4	74.7	0.32
2.	84.6	13.3	78.7	0.75	78.9	0.88
3.	86.4	10.1	84.3	0.58	83.2	0.48
4.	77.5	8 8	81.5	0.30	72.8	0.57
5.	84.9	12.7	82.3	0.75	78.9	0.42
6.	84.8	7.5	82.2	0.40	76.4	0.42
7.	83.1	9.7	81.3	0.28	80.9	0.22
Control	72.9	21.2	70.5	15.6	67.4	15.2
C.D.	5.1	5.0	10.4	3.0	9.0	1.1

G.P. = Germination percentage
D.P. = Disease percentage

TABLE VI

(December, 1946)

Germination and disease percentage of stored seeds

Days of sunning	Sunning		Compound I		Compound II	
	(G.P.)	(D.P.)	(G.P.)	(D.P.)	(G.P.)	(D.P.)
0.	0.0	0.0	0.0	0.0	0.0	0.0
1.	63.0	13 5	50.0	1.0	24.5	0.6
3.	70.0	15.4	54.0	0.0	85.0	0.0
4.	65.0	21.0	71.0	0.8	83.0	0.0
5.	88.0	2.8	76.0	0.0	89.5	0.0
6.	87.5	2.3	79.5	0.0	90.5	0.0
7.	91.5	3.2	82.5	0.0	90.0	0.0

NOTE.—Experiment carried out in sterilized soil in small pots. Those diseased seeds which did not germinate at all, could not be taken into account.

Moisture—The seed immediately after the harvest contains on an average 21.4 per cent moisture ; after 2 days of drying in the sun, it falls to 16.8 per cent ; after 3 days it falls further to 8.8 per cent and to 7.3 per cent average after 4 days.

From the above tables it is seen that the infection of the disease tends to be less as the hours of drying in the sun increase. With long storage this tendency is evident with stronger contrast. Probably the presence of higher amount of moisture helps the pathogen to remain viable in mycelial stage or to pass to the sclerotial stage during storage. A very significant feature is that the samples which were stored without drying, completely lost their viability, after 14 months of storing. Many such seeds on examination were found to be without hyphae or sclerotia. This indicates that presence of higher percentage of moisture caused the seeds to lose viability by upsetting some physiological balance and the complete loss of viability is not entirely correlated with the disease.

Drying in the sun. Solar energy plays an important part in its relation to the moisture content of the seeds. From Table VI it is seen that after 14 months of storage, the samples which were in the sun for one day also lose their viability considerably. This may be compared with the results in table III for samples with no sunning.

Although in the early months of storage, the influence of moisture content of seeds on viability is not apparent, it is evident however that retention of 'good' viability depends upon thorough drying in the sun.

Whether co-ordination of more factors is responsible for perfect dormancy or not is a point for further investigation.

Commercial dusts :—It will be seen that commercial dust, as mentioned here, is efficient in its fungistatic action which becomes more apparent by storing. Post treatment storing does not show any deleterious effect on the viability, atleast up to 14 months.

Treatment with compound I or compound II gives best result, when the seeds are dried in the sun for more than 5 days. The adverse effect of high moisture content is independent of treatment of such dusts, although the germination index for compound I with lesser hours of sunning, is inferior to that with compound II.

Seedlings from seeds treated with compound II appear stouter and more vigorous but shorter than the untreated ones, which are long and thin.

Those treated with compound I are stouter than the untreated ones but longer than those treated with compound II. The latter seem to have some retarding effects on germination.

CONCLUSIONS

1. Jute seeds are best stored after drying in the sun for a minimum period of 4 days or more, when the moisture content is on an average 7.5 per cent.

2. Treatment with organo-mercuric compounds gives additional beneficial results.
3. Seeds treated with these dusts can be stored for more than 14 months without any danger of their losing viability provided they are kept in air-tight moisture free, non-porus containers in dry places.

The cost of such treatment and storage is very small and can be profitably practised by jute farmers.

SUMMARY

Corchorus capsularis and *Corchorus olitorius* are the two commonly cultivated species of jute. Both of them suffer from 'stem-rot', primarily a seed-borne disease. The best remedy for controlling this primary infection is by the dry seed-treatment with commercial dusts. It is a common experience that the jute seeds cannot be stored in a healthy condition for a long period. The reason may be ascribed to the premature seed collection, the retention of high percentage of moisture through improper drying in the sun and fungal infection.

Hence proper drying, at least for 4 days, followed by treatment with organo-mercuric compounds is recommended before storage. Storage is best under dry, air-tight conditions. The cost for seed treatment is very low.

We express our sincere thanks to Dr. B. B. Mundkur, who has kindly gone through the manuscript and helped us with valuable suggestions.

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RED SPOT DISEASES OF LEAVES OF JOWAR (*ANDROPOGON SORGHUM* BROT.)

I, Ascochyta Leaf Spots*

R. S. Singh, P. R. Mehta and B. Singh

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Introduction

Of all the diseases of forage and grain crops grown in the Kharif season in India, 'red spots' on leaves of jowar and allied grasses are the most common. Though not so important from an economic point of view, they are widely distributed. Scattered records exist showing the association of a number of fungi with these spots on leaves of *Andropogon* spp. in America and Europe (Diehl 1938, Bain and Edgerton 1942, Bain 1945, Sprague and Johnson 1950).

In India, however, only a few pathogenic fungi have so far been reported to be associated with red spots of jowar leaves. Butler and Bisby (1931) reported *Colletotrichum graminicolum* (Ces.) Wilson and *Helminthosporium turcicum* Passerini as the cause of red spots on the leaves of *Andropogon sorghum*. Mundkur (1938) reported a leaf spot of *Andropogon sorghum* caused by *Cercospora sorghi* Ell. and Ev. in Bombay and Madras, Mehta and Bose (1946) reported the occurrence of *Titœospora andropogonis* (Miura) Tai on jowar at Kanpur. The disease caused by this fungus has been studied in the U.S.A. by Olive *et al* (1946) and the causal organism has been renamed as *Ramulispora sorghi* (Ell. and Ev.) Olive and Lefebvre.

The work on the fungi associated with leaf spots of jowar was initiated by the second author in 1945 in the Plant Pathological Laboratory of the Government Agricultural College, Kanpur and has been in progress since then. At Kanpur the fungi associated with red spots on jowar leaves have been found to be *Helminthosporium turcicum* Pass., *Colletotrichum graminicolum* (Ces.) Wilson., *Ascochyta sorghi* Sacc., *Glœocercospora sorghi* Bain and Edg., and *Titaeospora andropogonis* (Miura} Tai (=*Ramulispora sorghi*). This paper is the first of a series in which it is planned to give an account of some salient features of the organisms causing red spots on jowar leaves. The investigation presented below deals with certain etiological and pathological aspects of *Ascochyta sorghi* Sacc. found as the cause of one type of spots on the leaves of *Andropogon sorghum* at Kanpur.

OCCURRENCE

During the last several years the *Ascochyta* spots of jowar have been very severe at Kanpur, especially during heavy rains. These spots usually appear in the second week of July and the severity of their appearance is maintained upto the middle of September. Ill-drained, weed-

*Condensed version of a thesis submitted by the senior author for the M.Sc. (Ag.) degree of the Agra University.

infested fields, a temperature range of 20°C to 35°C and excessive humidity appear to be the chief contributory factors that favour the appearance and rapid spread of the disease.

SYMPTOMS

The spots generally appear on both the surfaces of the lamina, rarely on the midrib and leaf sheaths. They have not been observed on the stem and inflorescence. The appearance of these spots is preceded by a slight yellowing or fading out of the green colour. The spots are either elliptical (Pl.I, Figs. 1-4), 1.0 to 1.5 cm. by 0.5 cm. or elongated (Pl.I, Figs. 6-7), sometimes very fine, 1.0 to 3.0 cms. by 0.1 to 0.3 cm. The elliptical or broad elongated spots between the veins usually coalesce in later stages and form irregular spots of various dimensions (Pl. I, Fig. 5). The colour of spots varies from brick red or deep liver brown to warm blackish brown. However, different shades of brown are common on different types of spots. The central portion of very old spots usually becomes white or drab grey. Appearance of black, erumpent to superficial pycnidia on these spots, from the very beginning, is the chief feature of the disease.

The area of the leaf surface covered by the spots varies according to the type of spots described above. Generally in the case of broad, elongated spots as much as 15 percent. of the leaf area may be involved. The percentage of infected plants in fields varies from 10 to 15, and usually about 50 per cent of the leaves on a plant are found affected.

MORPHOLOGY AND PATHOLOGICAL HISTOLOGY

Within a day or two of the fading out of the green leaf colour, preceding the development of definite spots, dark brown or black pycnidia become visible on them. They are completely embedded in the leaf tissues at first but gradually erumpent and superficial pycnidia also appear on the same spots. During wet weather in the months of July and August a large number of spores are seen in these pycnidia. Microscopic examination of the spot shows the presence of septate, much branched, inter-and intra-cellular hyphae within the leaf tissue. The hyphae collect into dense, spherical, pseudoparenchymatous masses by gradually displacing the leaf tissues. The outer hyphae all round the pseudoparenchyma turn brown and form a loose peridium (Pl. II, Fig. 5). In a few cases this peridium around the dense masses is absent. These pycnidia remain embedded in the leaf tissues throughout. The superficial pycnidia are formed by the hyphae which collect in the epidermal or subepidermal cells, come out at many points and spread on the leaf surface in a thin mat. The superficial pycnidia invariably possess a definite hard wall (Pl. II, Fig. 1-4).

The pycnidia are mostly spherical and rarely possess an ostiole. They are light olive brown to black in colour under the microscope. Their diameter on the host varies from 204.7 μ to 338.2 μ with an average of 249.2 μ.

The pycnidia, in the presence of moisture, swell up, the wall becomes soft and disintegrates liberating the spores in surrounding film of water.

The spores (Pl. II, Fig. 6) are without any gelatinous matrix, mostly bicelled, sometimes unicellular when young, and rarely 3 to 4 celled They are ellipsoid, oblong or reniform in shape with obtuse or round ends ; usually straight or slightly curved and hyaline. Measurements made from 500 fresh spores from the host show their size varying from 13.3 to 19.9 μ in length and 4.2 to 6.6 μ in width with an average of 15.6 $\times$ 5.5 μ.

ISOLATION AND PATHOGENICITY

The fungus has been easily isolated on 2 per cent potato dextrose agar. It grows well on a number of media including oat meal agar, jowar leaf extract agar and nutrient glucose agar. Black, stromatic bodies, resembling pycnidia, develop abundantly in 5—7 days old cultures. However, spores have not been so far found to develop in these bodies on any of the media at room temperatures (20°C to 35°C). These pycnidia-like bodies are slightly bigger than pycnidia formed on the host. Their size on different media varies from 201.4 $\times$ 197.0μ to 310.6$\times$295.0 μ.

The pathogenicity of the isolates has been tested by placing hyphal fragment or pycnidia-like bodies on the surface of the leaf and keeping inoculated leaves in moist chamber for three days. Symptoms, identical to those caused by *Ascochyta sorghi* on jowar, are produced after 7 to 10 days. Isolations from these spots give cultures identical to the original ones.

Pathogenicity tests have also been carried out on leaves of young plants (2-week old) and mature plants by using scrapping from infected leaves which contained a large number of pycnidia. This inoculum was suspended in water and sprayed on plants of different ages. The inoculated plants were then incubated in moist chambers for 48 hours. In cases of successful infection the symptoms appear between 7 to 10 days after inoculation. The results are given in table I.

TABLE I

Results of inoculations on young and old plants of Andropogon sorghum *with pycnidial scrappings and spores of* Ascochyta sorghi *from host leaves as well as with mycelium and pycnidia-like structures from cultures.*

Source of inoculum	Young plants			Mature plants		
	Number of leaves inoculated	Number of leaves infected	Per cent leaves infected	Number of leaves inoculated	Number of leaves infected	Per cent leaves infected
One year old pycnidia from dry leaves	160	0	0	80	0	0
Fresh pycnidia from leaves	175	89	51	190	109	57
Spores from pycnidia	125	61	49	199	112	56
Mycelium and pycnidia from cultures	121	29	24	195	52	26
Check	127	0	0	127	0	0

Inoculation of plants with one year old pycnidia has given negative results. Fresh pycnidia produce a high percentage of infection. There are definite indications that the leaves of young plants are generally more resistant than leaves of older plants.

SPORE-GERMINATION

The spores readily germinate in a film of water or nutrient solutions. At first vacuoles appear in the segments of the spores. The cell protoplasm grows out into a small, thick protuberance from the apex or any side of the spores. Usually the germ tubes are produced from both the segments of the spore. Production of two germ tubes, one from each segment is most common but the spores may produce several germ tubes from either of the segments. Sometimes the segments become swollen and spherical before germination.

At room temperatures (30°C - 35°C) the spores commence to germinate after two and a half hour in different media*. During a period of 24-hour germination test at 30°C to 35°C., the germination percentage has been found to be higher in one percent glucose solution as compared to other media tried. At temperatures mentioned above, about 98 per cent of spores germinate within 48 hours in different concentrations of glucose solution and in leaf extract. Apart from the percentage of germination, the length of the germ tubes is also affected by the various media. The maximum length of the germ tubes is obtained in glucose solutions and in extracts of jowar leaves and seeds.

Good germination of spores can also be obtained on dry slides provided the slides are kept in atmospheres having 97 to 100 per cent relative humidities. During a 48-hour germination test it has been found that the spores do not germinate in relative humidities below 85 per cent. Even in 90 per cent relative humidity only about 6 per cent of the spores germinate in 48 hours, but in atmospheres with higher moisture contents (97 to 100 per cent relative humidities) 60 to 98 per cent of the spores germinate within 48 hours.

To ascertain the effect of temperature on spore germination the spores were placed in hanging drop cultures at temperatures varying from 8°C to 40°C. The results of these tests are given in table II.

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* Media on which spore-germination studies were made are :—
Distilled water ; sterile tap water ; ordinary tap water ; 0.5, 1.0, 2.0 and 7.0 per cent glucose solutions, and extracts of jowar leaves and seeds.

TABLE II

Germination percentage of spores of **Ascochyta sorghi** *at different temperatures.*

Temperature (°C.)	Percentage of germination after			
	6 Hours	12 Hours	24 Hours	48 Hours
40	0.0	0.0	0.0	0.0
38	28.0	55.0	64.0	80.0
35	33.0	71.0	82.0	94.0
32	36.0	78.0	89.0	96.0
30	35.0	79.0	89.0	96.0
28	27.0	68.0	83.0	93.0
25	0.0	2.0	6.0	17.0
20	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0

During these tests it has been found that the spores do not germinate at and below 20°C. and above 38°C. The minimum, optimum and maximum temperature for germination of these spores is thus 25°C., 30°-32°C. and 38°C.

VIABILITY

The viability of the mycelium, pycnidia and the spores is not affected at 4°C. when diseased leaf pieces are kept for three to seven days in a refrigerator. The mycelium in cultures dies within 24 hours at temperatures above 42°C. The pycnidia on the diseased leaves resist a temperature of 48°C. for at least 36 hours but are killed at 50°C. within 8 hours. The spores were exposed to different temperatures for definite durations to ascertain the thermal death point. The results are shown in table III.

TABLE III

Effect of exposure of spores to different temperatures for various durations on their viability

Temperature (°C.)	Percentage of germination of spores at room temperature in 48 hours			
	Period of exposure to different temperature in minutes			
	1	2	5	10
38	98	98	97	97
40	98	97	97	96
43	96	94	91	86
46	93	92	90	81
48	69	54	9	0
50	0	0	0	0

These figures give definite indications that the viability of spores exposed to 38°C. and 40°C. for 1 to 10 minutes is not appreciably affected. Spores exposed to 43°C and above for 2 minutes to 10 minutes show a marked reduction in their germination percentage and they are killed within 10 minutes when exposed to 48°C.

In periodical germination tests of the spores from pycnidia on leaves stored in the laboratory, (temperature range 20°C. to 35°C.), the spores have been found to lose their viability within 6 months although the fungus could easily be isolated from spots on such leaves even after a year.

HOST-RANGE

The fungus has been found to cause similar leaf spots on *Andropogon halepensis* and *Andropogon sorghum* var. *sudanensis* at Kanpur. The spore measurements and cultural characters of the isolates from these collateral hosts are identical to those of the *Ascochyta* from *Andropogon sorghum*. Cross-inoculations have given additional evidence as to their being identical fungi. The results of these cross-inoculation experiments are given in table IV. These collateral hosts, which are perennial grasses, also serve as an important source of perpetuating and spreading the disease.

PLATE I

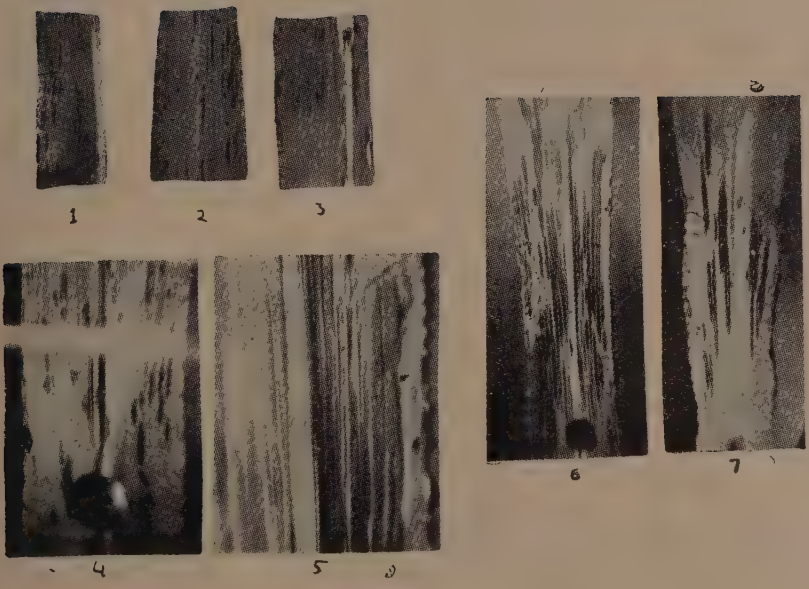

TABLE IV

Percentage of infection on leaves of Andropogon *spp. caused by isolates of* Ascochyta sorghi *from different hosts*

Spores taken from	Species on which inoculated	Percentage of infection	Incubation period (days)
Andropogon sorghum	*Andropogon sorghum*	65.0	7–8
	Andropogon halepensis	70.0	7–8
	Andropogon sorghum var. *sudanensis*	10.0	8–9
Andropogon halepensis	*Andropogon sorghum*	60.0	7–8
	Andropogon halepensis	92.0	8–9
	Andropogon sorghum var. *sudanensis*	23.3	9–10
Andropogon sorghum var. *sudanensis*	*Andropogon sorghum*	15.0	7–8
	Andropogon halepensis	42.5	7–8
	Andropogon sorghum var. *sudanensis*	60.0	7–8

PERPETUATION AND SPREAD OF THE DISEASE

Seeds collected from diseased and healthy plants have been tested for external or internal presence of the fungus. The experiment was conducted in two sets separately for seeds from healthy as well as diseased plants. In each set the following treatments were followed :—

1. Seeds plated as such.
2. Seeds washed thoroughly with sterile water before plating.
3. Seeds washed with 0.1 per cent mercuric chloride solution, followed by three washings with sterile water.

The seeds were plated on water agar in Petri dishes. In all 60 seeds were plated for each treatment and the experiment was repeated three times. Thus for each treatmant 180 seeds were examined. In no case, however, cultures of *Ascochyta sorghi* could be obtained. It appears, therefore, unlikely that the disease is seed-borne.

Since the disease has not been found to be seed-borne in nature and the spores lose their viability within six months of their production, it appears likely that the perpetuation of the disease is accomplished by viable mycelium and pycnidia present in diseased leaf trash in fields. Experiments have been carried out to ascertain the role of mycelium and pycnidia left in fields as such or in diseased trash. Sterilized soil was infested with fungus inoculum and seeds of jowar were grown in it. The results are shown in Table V.

TABLE V

Percentage of infection caused by one year old inoculum of Ascochyta sorghi *kept under laboratory as well as under field conditions.*

Expt. No.	Treatment	Percentage of infection (based on 25 plants)
1.	Sterilized soil inoculated with one year old pycnidia that were kept in sterilised tubes in laboratory (20°C. to 33°C.)	52
2.	Sterilized soil in pots inoculated with fresh pycnidia and kept exposed outside for a year before sowing.	64
3.	Sterilized soil inoculated with one year old trashes of diseased leaves that were kept in laboratory (20°C. to 33°C.)	80
4.	Sterilized soil in pots mixed with fresh pieces of diseased leaves and kept outside for one year.	100
5.	Sterilized soil inoculated with one year old cultures of the fungus grown on maize meal soil medium.	60

N.B.—No disease appeared in control pots.

Definite indications as to the disease being soil-borne have been obtained by the experiments mentioned in Table V. Whether the sterilised soil is inoculated with one year old pycnidia or diseased leaf trash just before sowing or the infested soil is exposed to weathering for a year the results are always more or less identical and successful infection has been obtained.

Secondary infection of the healthy leaves and spread of the disease takes place through the spores liberated by disintegration of the pycnidial wall during a continuous drizzle. They are transmitted through the agencies of water, wind and insects. The moistened pycnidia easily adhere to

anything that passes through the infected fields. Microscopic examinations of grasshoppers trapped from infected fields showed the presence of pycnidia adhering to the pulvillus or the leg and also on other parts of their body. In dry weather, however, no pycnidia were found on the body of these insects.

DISCUSSION

Jowar is one of the most common fodder crops of India. It is grown for fodder as well as for grains. The acreage under both fodder and grain crops in the Uttar Pradesh is about two and a half million acres. The fodder crop is usually sown thick (12–15 seers seed per acre), whereas the crop for grains receives better care and is sown comparatively thin (4 to 5 seers per acre). On account of the plants being closely crowded, the resulting fodder crop is more tender and due to dense growth there are better chances for spread of infection of various disease-causing fungi. On the other hand the grain crop sown with spacing shows a more vigorous growth and is consequently less liable to attack by the fungi. It has been frequently noticed in fields that 'Ascochyta spots' are more common in fodder crops than in grain crops. The spread of this disease, as also is the case with other red spots diseases of jowar, is very rapid in thickly sown fodder crops during wet weather. This is apparently due to close contact of diseased and healthy leaves resulting from a dense stand, and the presence of moisture on leaves for a longer duration. Furthermore, insects, like grasshoppers are always most frequent in such dense crops and are one of the agents of carrying infective material from leaf to leaf and from field to field.

The fodder crop is cut from the base of the stem when young and fed to cattle. Usually very little leaf trash is left in the field after the crop has been removed for consumption as fodder. Under these conditions it is unlikely that too much of fungus inoculum is left with diseased leaf trash in the field. Even then the disease appears always more severely in fodder crops than in grain crops. However, some of the older leaves near the stem-base fall on the ground and are left in the field to carry over the fungus. The superficial pycnidia, in relatively drier weather, readily fall down on the ground and may survive in the soil till the next sowing season. Though one-year-old pycnidia have been found to be unable to infect leaves when sprayed with water, they have been able to do so when mixed with soil before sowing of the crop.

The fungi that have been found associated with red spots of jowar, at Kanpur, such as *Titæospora andropogonis* (=*Ramulispora sorghi*) and *Glœocercospora sorghi* develop sclerotial storma to resist adverse weather and survive in the soil. The fungus under study also develops abundant pycnidia that are very hard and can resist fairly high temperatures for considerably long periods. The possibility that farm yard manure may serve as a medium for carrying the pycnidia to fields and cause primary infection has not been explored, but it is likely that the pycnidia fed to cattle along with green leaves may servive the elimentary canal of the cattle and pass out with the dung without losing their viability. The manures may thus carry the infection to healthy fields.

However, the most important source of perpetuation and spread of the disease seems to be the perennial grasses (*Andropogon halepensis* and *Andropogon sorghum* var. *sudanensis*) mentioned in this paper. *Andropogon halepensis* is one of the most common perennial grasses growing in this locality. They are common in the jowar fields. The infection, under favourable conditions, on this grass is in no way less than on jowar. With the onset of monsoons these grasses renew their leafy growth and with them appear the *Ascochyta* spots with abundant pycnidia bearing numerous spores. By the time, jowar is sown in fields, these grasses have produced enough of infective material to be disseminated by wind and rain.

Whatever the source of introduction of the inoculum into the fields may be, it has definitely been proved that the disease is soil-borne and primary infection occurs through soil. The pycnidia and mycelium in diseased trash can normally survive the summer temperature of the soil. It has been noticed during field observations that the disease appears earliest and is most common in the neighbourhood of those fields which had a diseased crop of jowar in the preceding year and where the growth of *Andropogon halepensis* is common on the borders of the fields and irrigation channels.

SUMMARY

Ascochyta sorghi, hitherto unrecorded from India, has been found associated with red spot diseases of *Andropogon sorghum*, *Andropogon sorghum* var. *sudanensis*, and *Andropogon halepensis* at Kanpur. The spots vary from light brown to deep brown in colour, and streaks to large elongated and broad patches in shape and size.

Microscopic study of the fungus shows that the fruiting body consists of light brown to black coloured pycnidia which are either embedded in the leaf tissues or are superficial, and contain numerous spores.

In cultures the fungus produces black stromatic pycnidia-like bodies but they never produce spores. The mycelium and the pycnidia-like bodies in cultures are able to infect jowar leaves. One year old pycnidia from host leaves are unable to cause infection when sprayed with water but they produce the disease when mixed with sterilised soil in pots.

The minimum, optimum and maximum temperatures for spore-germination have been found to be 25°, 30°-32°, and 38°C. The mycelium, pycnidia and spores can withstand freezing temperatures for short periods. The mycelium in cultures dies within 24 hours at temperatures above 42°C. The pycnidia can survive a temperature of 48°C., for at least 36 hours but at 50°C. they are killed within 8 hours. The spores die within 10 minutes at 48°C. In nature viability of the spores is lost within 6 months.

The disease is perpetuated through the mycelium and pycnidia in soil or in diseased leaf trash left in the field. The spread of the disease is by pycnidia and spores through the agencies of wind, water, and insects. The

presence of perennial grass hosts such as *Andropogon halepensis* plays an important role in perpetuating and spreading the disease.

Plant Pathological Laboratory
Government Agricultural College
Kanpur

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PLATE II.

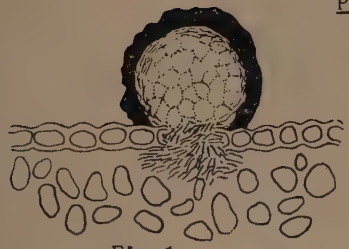

Fig.1

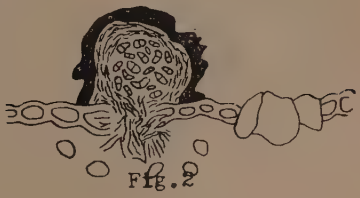

Fig.2

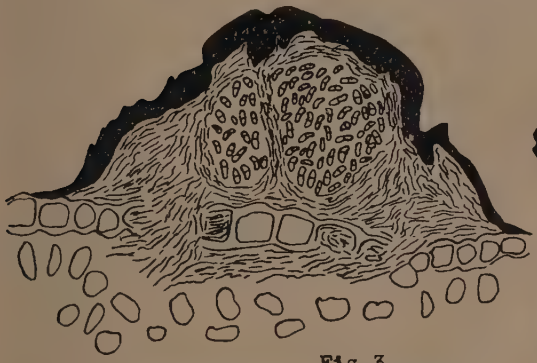

Fig.3.

Fig.5

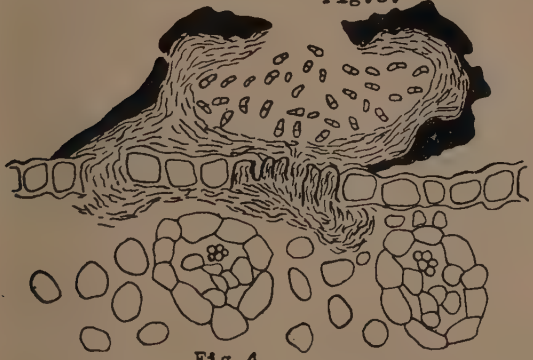

Fig.4

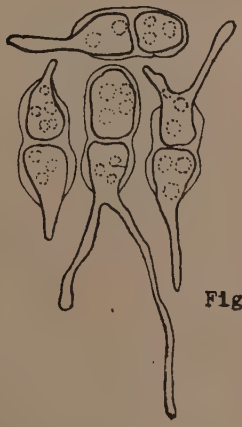

Fig.7

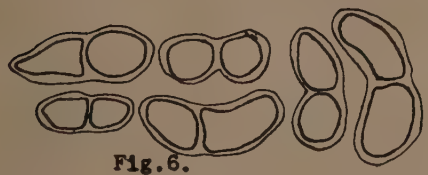

Fig.6.

BACTERIAL SOFT ROT OF ONIONS IN STORAGE

By

M. K. HINGORANI AND D. S. MALLA[1]

(Accepted for publication May 31, 1951)

During the last two years, rotting onion bulbs have been received in the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, for examination. Majority of these diseased bulbs had their inner scales watersoaked and soft and they emitted a foul odour. Some of them appeared sound on the outside, but, when cut open, one or two scales were found to be infected. Microscopic examination of the affected portions showed them to be full of bacteria.

Several workers in other countries have reported bacterial rots of onions describing a number of bacteria as causing or at least associated with the disease. Burkholder (1942, 1950) has shown that two new species of bacteria, namely, *Phytomonas* (*Pseudomonas*) *alliicola* and *Pseudomonas capacia* are responsible for onion rot, the latter organism causing a characteristic rot known as 'Sour Skin'. Search through Indian literature, however, revealed no reference to soft rot of onion bulbs. This paper forms a detailed account of one such record.

Isolation : The pathogen was readily isolated in pure culture from the interior of the bulbs along the margin of the farthest advance of the decay. Dirty white bacterial colonies appeared on the dilution plates. These colonies were characterized by their ability to colour beef-extract-peptone broth a deep green. In describing this bacterium 6 isolates, each from a separate onion bulb, were used. Each isolate was replated and single colony cultures obtained prior to each series of tests. The only difference observed among the isolates was in the intensity of the green fluorescent pigment produced.

Pathogenicity : Inoculations with the onion organism, made on a number of vegetables, produce a yellowish-green rot on onions, carrot, potato, lettuce and its related plants ; beet-root, brinjal, cabbage, cauliflower, cucumber, kohl-rabi, chillies, radish, tomato, and turnip are however not attacked.

Morphology : The onion pathogen is a short rod with rounded ends, single or in chains of two, and has no involution forms. It is motile by one to three polar flagella, Gram-negative, capsulated, not-acid-fast, and nonspore-former. The average dimensions of bacteria, from one week old cultures, are $2.2 \times 0.8\ \mu$. It readily stains with carbol fuchsin, gentian violet, methylene blue, victoria blue and bismarck brown.

1. The senior author is Assistant Plant Bacteriologist in the Mycology Division of the Indian Agricultural Research Institute, New Delhi. The junior author was a post-graduate student in the same Division when this work was done.

Cultural Characters : Streak cultures on nutrient agar are creamy to yellowish-green, moderate in growth, spreading, not adherent, articulate and glistening. Medium is discoloured from light to deep green. In nutrient broth, surface growth is abundant and turbid with the formation of pellicle. Green fluorescent pigment, first produced near the surface, extends downward into the tube. Growth on potato-dextrose agar is abundant, filiform, glistening, smooth, raised, white to dirty cream with no distinctive odour. Growth on potato cylinders is copious, greenish-yellow, raised, smooth and shining. In Uschinskey's medium, referred to as No. 463 by Levine and Schoenlein (1930), and Clara's solution (1934), green fluorescent pigment is produced which within 12 days changes to yellowish-green. There is no growth in Cohn's solution. The pathogen is an aerobe. The optimum temperature for growth is 25° to 28° C., growth ceasing at 3° and 40° C. The thermal death point is about 50° C.

Biochemical Reactions : Growth in gelatin stabs is good, liquefaction begins on the second day and is rapid. Nitrates are reduced to nitrites and nitrogen. In litmus milk, a soft coagulum is formed, with rapid peptonization and reduction of litmus ; reaction is alkaline in 48 hours. Starch is not hydrolysed and indole, hydrogen sulfide or ammonia are not formed. The organism shows good lipolytic activity. M. R. and V. P. testsare negative. Acid (without gas) produced from dextrose, glycerol and mannitol, but not from levulose, galactose, arabinose, maltose, lactose, sucrose and salicin.

Identity : It is clear from the data presented above that the onion organism is pathologically, morphologically, and physiologically related to *Pseudomonas marginalis* (Brown) Stevens. Dowson (1941) also reports that this organism is responsible for certain types of rots in stored potatoes in England and causes a rapid decay of onions as well.

The writers are extremely thankful to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, for valuable suggestions made during the progress of this investigation and for reading the manuscript.

Division of Mycology and Plant Pathology
Indian Agricultural Research Institute
New Delhi

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SEEDLING BLIGHT OF RICE IN SIND

BY

M. K. HINGORANI[1] AND N. PRASAD[2]

(Accepted for publication June 1, 1951)

INTRODUCTORY

The rice crop in Sind suffers from a number of diseases of which 'brown spot' caused by *Helminthosporium oryzae* Breda de Haan [*Cochliobolus miyabeanus* (Ito & Kurib.) Drech. ex Dastur] is the most serious. The disease first attracted attention in 1925 when it appeared in an epidemic form, destroying more than 50 per cent of the crop. The loss of 1 to 5 per cent every year is, however, not uncommon.

The disease manifests itself in two stages—(1) a seedling phase due to infection from seed causing root and foot-rot of seedlings ; and (2) a secondary phase when small brown spots appear on the leaves and ears. In severe cases the plants get stunted, the spots get numerous and run together, and sometimes the entire leaves wither. Whole fields in such cases present a characteristic burnt and scorched appearance.

A review of literature on blighting of rice seedlings is given by Cralley and Tullis (1937) and recently by Padwick (1950). It has been shown that besides *Helminthosporium oryzae*, several other fungi are responsible for this trouble. In India, Sundararaman (1922) first reported the disease and gave a description of its causal agent on the basis of which Mitra (1931) identified the fungus as *Helminthosporium oryzae.* Later, Thomas (1931, 1933) observed a severe case of foot-rot of rice seedlings in Madras, which he attributed to *Gibberella fujikuroi* (Saw.) Wr. The two disorders, however, differ from one another in symptomatology.

The present study deals with the cause of seedling blight of rice in Sind and presents the effects of seed treatment by chemicals on its severity.

FUNGI ASSOCIATED WITH BLIGHT

An intensive survey was made of the rice-growing tracts and diseased specimens were collected from several localities. More than 500 isolations were made from discoloured leaves, kernels, and seedlings just above the kernels, by immersing small pieces in a 1 : 1000 solution of bichloride of mercury for 1 to 2 minutes followed by a thorough washing in sterilized water. They were then cultured in Petri dishes containing oat-meal agar and incubated at 30°C. for one week. Table I shows the occurrence of the most prevalent types of fungi obtained.

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[1] Now at the Indian Agricultural Research Institute, New Delhi
[2] Now at the Agricultural Institute, Anand

TABLE I

Relative prevalence of fungi obtained from the diseased kernels, leaves, and seedlings of rice

Source	Percentage prevalence of fungi isolated			
	C. lunata	H. oryzae	Helminthosporium sp.	Other fungi
Kernels	60	30	8	2
Leaves	15	55	26	4
Seedlings	50	38	10	2

It will be noted that *Curvularia lunata* and *Helminthosporium oryzae* were the most frequently isolated fungi. *Helminthosporuim* sp. differed from *Helminthosporium oryzae* in spore size and number of septa, the former having smaller spores and fewer septa. The other fungi included spp. of *Aspergillus*, *Rhizopus* and *Alternaria* which were discarded, as they were non-pathogenic.

PATHOGENICITY

Seed of a susceptible variety of rice (*Kangni* 27) was used for the pathogenicity tests. Healthy grains were first surface-sterilized in 1 : 1000 mercuric chloride solution for one minute, washed in sterile water, and dried by spreading evenly in a sterilized Petri dish. They were then immersed in a spore suspenion of the desired fungus for about half an hour and sown in sterilized soil in medium-sized pots. Temperature during the experiment ranged from 38° to 40°C. Controls did not receive any fungal treatment. The results are presented in Table II.

TABLE II

The results of inoculation with the three fungi isolated

Pot No.	Percentage of infected seedlings per pot			
	C. lunata	H. oryzae	Helminthosporium sp.	Control
1	64	72	43	0
2	60	56	48	0
3	80	80	72	0
4	72	44	24	0

EFFECT OF DUST TREATMENT

There are several reports on the effects of seed disinfection by chemicals for the control of seedling blight, but the results reported are not always uniform. Cralley and Tullis (1937) and Padwick (1950) have reviewed earlier work in considerable detail to which a reference has been made.

Pot-culture and field experiments were, therefore, laid out to determine the effects of Semesan, Ceresan, Granosan, Uspulun, red-copper-oxide and Agrosan dusts, at the rates recommended by the manufacturers.

The seed lots, each consisting of 100 blighted seeds, were thoroughly mixed with the dust in small vials, after which the excess was removed by shaking the seed on a wire screen. The treated seed was placed in closed containers for 24 hours and sown. All the field operations were carried out according to local practices. There were three replications and observations were taken on 200 seedlings in each case, selected at random. Control included diseased seed without any dust treatment. The temperature ranged from 38° to 40°C. Data are summarized in Table III.

TABLE III

The effect of six dust disinfectants on the severity of seedling blight

Disinfectant	Mean percentages of seedling blight	
	Pot-culture	Field
Semesan	3	4
Granosan	5	4
Uspulun	14	6
Red-copper-oxide	6	9
Ceresan	4	7
Agrosan	2	3
Control	29	31

DISCUSSION

Curvularia lunata, *Helminthosporium oryzae*, and a *Helminthosporium* sp. were the fungi most consistently isolated from the diseased rice seedlings, leaves and kernels. It has been shown that the three fungi are responsible for seedling blight of rice in Sind.

The *Helminthosporium* sp. resembled in all its major characters, except in spore-size and number of septa, to *Helminthosporium oryzae*. The conidia of *Helminthosporium oryzae* measured 40-102 × 13-18 μ and were 5 to 10 septate. The spores of *Helminthosporium* sp. averaged 48 μ (25-70 μ) in length and 11 μ (8-13 μ) in width and the average number of septa was 5 (2-7).

There is some variation in the spore measurements of *Helminthosporium oryzae* from material collected in other parts of the world and even in the same country. Nisikado and Miyake (1922) showed that the Japanese strain varied greatly in length and breadth of conidia. They found the range of conidial measurements to be 10 to 26 μ in breadth and 15 to 132 μ in length. It appears safe, therefore, to group the organism into *Helminthosporium oryzae* or at the most consider it as a new variety of the same.

The results of experiments on the effects of seed treatment on the severity of blight have given good indications. It was found that in most cases the percentage of seedling blight was considerably reduced. The writers therefore conclude that seed disinfection by chemicals can be recommended for the control of seedling blight. For controlling secondary infection breeding of resistant varieties seems to be the best remedy.

SUMMARY

1. *Curvularia lunata*, *Helminthosporium oryzae*, and a small-spored *Helminthosporium*, probably a new variety of *Helminthosporium oryzae*, have been found to be associated with seedling blight of rice in Sind.

2. It has been shown that seed disinfection by chemicals can control the disease.

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ADDITIONS TO FUNGI OF BOMBAY-I

M. K. Patel, V. P. Gokhale and N. B. Kulkarni

(Accepted for publication June 29, 1951)

1. *Hemileia pavetticola* Maubl. and Roger in *Bull. Soc. mycol. Fr.* **54** 48-54, 1938

On *Pavetta indica* L. Mahableshwar, January, 1951, leg. M.K. Patel and V. P. Gokhale

A collection of a rust on the leaves of *Pavetta indica* proved on examination to be a sp. of *Hemileia.* The rust formed minute sori covering the underside of the leaves and were chiefly restricted to the lamina. Both the uredia and the telia were present ; the urediospores measured 17-29 × 14-22 μ and the teliospores 19-36 × 12-30 μ. The teliospores were triangular or crescentic and several of them showed early stages of germination.

Comparative studies indicated that the species under study should be referred to *Hemileia pavetticola* recorded on *Pavetta ternifolia* from Belgian Congo. *Hemileia mildbraedii* Syd. also occurs on *Pavetta oliveriana* in Central Africa, but the spore characters are different from those of *Hemileia pavetticola.* Further, the sori are formed on stems and inflorescence in *Hemileia mildbraedii.* Thirumalachar and Narasinhman (1947) studied the *Hemileia* spp. occurring on Rubiaceae in Mysore, but their collection did not include the rust under study.

2. *Uredo terminaliae* P. Henn. in *Hedwigia,* 1896

On leaves of *Terminalia chebula,* Mahableshwar

December, 1950, leg M.K. Patel and V. P. Gokhale

This rust in its uredinial stage was observed on *Terminalia chebula.* The uredia are hypophyllous, pulverulent and bright yellow. The urediospores measure 25 × 18 (20-30 × 15-21) μ. Telia were not observed even on dried leaves. The rust on this host agrees in all respects with *Uredo terminaliae* recorded on *Terminalia argentea* in Brazil.

3. *Ustilago deserticola* Speg. *An. Mus. Nac. de Buenos Aires* **6.** 209, 1898

On *Chloris villosa,* Surat, leg. M.K. Desai, 1947

The smut attacks the inflorescence converting it into a malformed structure. The sori are usually hidden and do not breakdown at maturity. Comparative studies indicate that it should be referred to *Ustilago deserticola* described from Argentina on *Chloris* sp., the spores being olive red, smooth and 5-8 μ in diameter. *Ustilago valentula* Syd. already recorded on *Chloris* sp. in India is a different smut species.

4. *Tolyposporium andropogonis* sp. nov.
On *Andropogon triticeus* R. Br. leg. M.K. Patel and N.B. Kulkarni, Poona, October, 1950

The smut entirely destroys the inflorescence converting it into a linear structure. The spore balls are firm and not breaking, the ridges of the epispore which help in binding them firmly with one another, can be made out. The presence of these connecting ridges on the epispore helps to separate species of *Tolyposporium* from *Sorosporium* which also often possesses spore balls rather firmly united. The type of sorus formation, either in the ovary or in the inflorescence is determined by the mode of infection of the plant in particular species concerned. The comparative studies with the descriptions of *Tolyposporium* and *Sorosporium* species recorded on the Andropogoneae have indicated that the smut under study has not been described before.

TOLYPOSPORIUM ANDROPOGONIS, Patel and Kulkarni sp. nov.

Sori in the inflorescence, entirely destroying it, covered by a cinnamon brown pseudomembrane, dehiscing irregularly and exposing the mass of black spore balls ; columella central and breaking into shreds. Spore balls ovate to spherical, 45 to 93 μ in diameter, less than 50 spores in a ball, bound together by the ridges developing on the spore wall appearing as papillate processes ; outer layer of spores reddish-brown, thick-walled, angular pue to lateral compression, smooth on the contiguous side and pitted and rugose on the free surface, 9 to 14 μ in diameter with a mean of 11 μ. : inner spores subglobose to spherical, pale cinnamon yellow to hyaline, smooth, 8 to 15 μ with a mean of 11 μ. Length of sorus ranging from 0.7 to 5.7 cm. Number of shreds in a sorus ranges from 6 to 12.

Hab. in the inflorescence of *Andropogon triticeus* R. Br. collected at Poona, in October, 1950.

TOLYPOSPORIUM ANDROPOGONIS, Patel and Kulkarni sp. nov.,

Sori in inflorescentia, quam penitus destruunt, operti pseudo-membrana cinnamomo-brunnea, irregulariter dehiscentes, exposita sporarum massa nigrarum, columella centrali in frustula rumpente. Sporarum massae ovatae ad sphaericas, 45-93 μ. diam. ; sporae pauciores quam 50 in singulis massis, simul retentae jugis in sporarum parietibus apparentibus, quae ut processus papillosi apparent ; sporae in serie exteriori luteo-brunneae, crasse voliatae, angulares ob compressionem lateralem, leves in latere contiguo, foveolatae atque rugosae in facie libera, 9-14μ diam., mediet, 11 μ. ; sporae interiores subglobosae ad sphaericas, pallide cinnamomo-luteae ad hyalinas, leves, 8-15 μ., mediet. 11 μ. Sorus 0.7—5.7 cm. longus ; frustula in singulis soris 6-12.

Habitat in inflorescentia *Andropogonis triticeus* R. Br., typus lectus in urbe Poona, mense octobri 1950.

5. *Balansia andropogonis* Syd.

On *Aristida* sp. leg. M. K. Patel and N. B. Kulkarni, Poona, October, 1950.

The diseased plants are dwarfed and malformed. The inflorescence is deformed in that the spikelets are replaced by leafy shoots. The floral parts are rudimentary or completely suppressed. Some rudimentary anthers with few pollen grains have been noticed. In general appearance, the malformed inflorescence appears as that incited by species of *Sclerospora.*

Sections through the infected spikelets reveal the delicate stromatic layer lining the inner surface of the sheathing leaves of the inner core of the malformed structure. The axis of the inflorescence often shows the stromatic layer of delicate hyphae. Numerous acicular conidia borne on short conidiophores, typical of *Ephelis* are present. In details of the spore, sorus formation and inciting sterility the fungus is similar to that reported by Diehl (1930) from U.S.A. Sclerotoid stage of the fungus is not known.

As regards the identity of the fungus, it may be tentatively considered as the conidial stage of *Balansia andropogonis* Syd. recorded in India on *Andropogon aciculatus* by Butler and Bisby. No conidial stage has so far been recorded for *B. andropogonis* and Sydow's statement that *Ephelis pallida* Pat. in Tonkin and the Philippines may probably be the conidial stage is only a surmise. Several of the *Ephelis* species on grasses and cereals are able to perennate in their conidial stages alone, the *Balansia* stage being either absent or of rare occurrence.

Thanks or due to Dr. M. J. Thirumalachar for help in determining the fungi and to Rev. Fr. H. Santapau for latin diagnosis.

Plant Pathological Laboratory
College of Agriculture
Poona

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A NEW SPECIES OF CERCOSPORA ON *GREWIA ASIATICA* LINN.

By

H. C. Srivastava and P. R. Mehta

(Accepted for Publication, June 29, 1951.)

During 1949-50, a severe leaf spot disease of *Grewia asiatica* was observed at the Botanical Garden, Kanpur. The disease was present throughout the year, but in June to August, its severity was at maximum, a majority of the leaves being infected. The disease was also observed at several other places of Uttar Pradesh. Preliminary microscopic examination revealed the presence of a species of *Cercospora.* As no species of *Cercospora* has hitherto been recorded on *Grewia asiatica*, further investigations were conducted.

The disease becomes manifest as tiny lesions on the dorsal and ventral side of the leaf ; these lesions are covered with a white crust of the fungal growth. Such fungal patches rapidly enlarge in diameter and become blackish in the centre. In due course, this black growth acquires a cushion like appearance. Such erumpent, cushion-like spots, are numerous all over the leaves, varying in thickness upto 0.5 mm. (Fig. 1). They are irregular and may coalesce to form big spots. The most notable feature of this species of *Cercospora* is its extra-matrical growth all over the leaf area and such growth consists of masses of conidia, conidiophores and superficial mycelium.

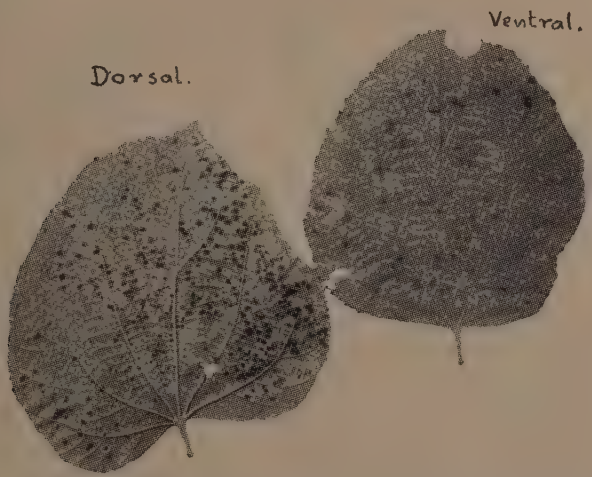

Fig. 1. Leaves of *Grewia asiatica* Linn. showing symptoms caused by *Cercospora grewia* sp. nov.

Mycelium of the fungus, which is mostly superficial, is septate, measuring 1.5 to 2.5μ in width. The hyphae have also been noted in the epidermal layer and are rarely seen in tissues more deeply seated. The mycelium which is white in the beginning, turning black later on, is

studded with numerous stroma consisting of thick walled cells, from which the conidiophores arise in bunches or tufts. The latter are generally simple but occasionally branched, several septate, dark coloured, 58-106μ long and 2.8—3.8μ wide with smooth walls. The knee joints are numerous and minute but distinct. Conidia which are attached on such joints, are tapering towards each end, with a long pointed and more acute apical cell. Conidia are hyaline, generally 1-6, mainly 2-4 septate, measuring 28-52μ in length and 4.8μ in width. They are curved in the centre. The upper end (apical cell) measures 7-36μ in length. (Fig. 2).

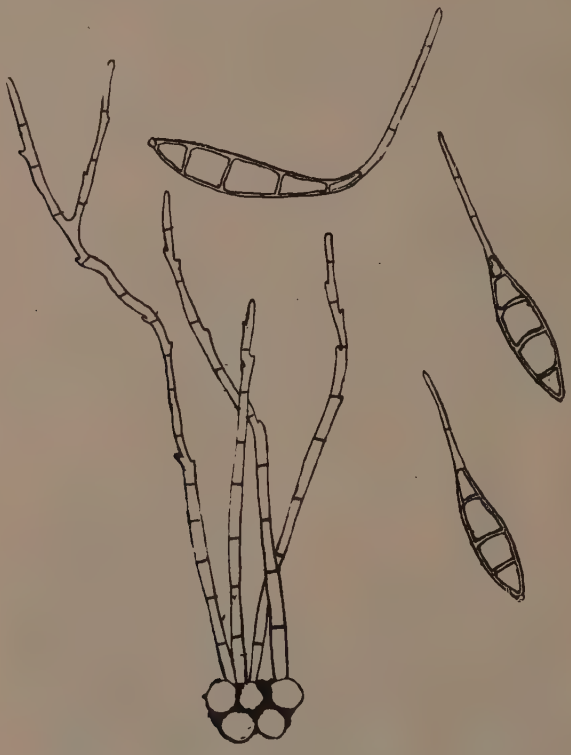

Fig. 2. Showing Conidia and Conidiophores of *Cercospora grewiae* sp. nov, × 500

The conidia germinate rapidly in water by the production of a germ tube from every segment excepting the capital cell. All efforts to grow the fungus in the artificial media has so far failed, (in Coon's synthetic medium, Potato dextrose agar medium and *Grewia* leaf extract agar).

Inoculation experiments were conducted during humid as well as dry weather on a large number of leaves. It was found that in the moist weather the inoculation experiments were quite successful and the fungus grew on the surface of the leave with a cushion-like growth, within five days after inoculation.

The diagnosis of the fungus was compared with that of the species reported previously on other species of Malvaceae and Tilliaceae. Since the fungus occurring on *Grewia asiatica* appeared to be a distinct species, specimens were sent to Royal Botanical Gardens, Kew (England) for further confirmation. The Director of those gardens reports that the species of *Cercospora* is "apparently undescribed". The fungus closely resembles *Helminthosporium grewiae* P. Henn. described from Congo.

Cercospora grewiae, Srivastava and Mehta, sp. nov.

Spots regular or irregular, more or less round, epiphyllous, gregarious, frequently involving large areas of the leaves, black cushion-like growth. Mycelium mostly external, white in the beginning then turning black, 2-3μ wide. Conidiophores effused on the mycelium, arising from a loose stroma, amphigenous, fasciculate, moderately or densely tufted, simple or occasionally branched, walls smooth or irregular, 58-106μ long, 2.8-3.8μ wide, many septate, dark coloured; conidial scars minute and distinct. Conidia fusoid, somewhat curved in the centre, the upper end tapering acutely, 7 to 36μ in length, generally 1 to 6, mainly 2 to 4 septate, 28 to 52μ long and 4 to 8μ wide.

Habitat: On living leaves of *Grewia asiatica* Linn. at Kanpur, U.P., India.

Cercospora grewiae, Srivastava and Mehta, sp. nov.

Maculae regularis vel irregulares, plus minusve circulares, epiphyllae, gregariae, saepe amplam aream foliorum occupantes, in mortuis vel sanis partibus, nigrrae pivillo similes crescentes. Mycelium externum, initio album, denum nigrumevadens, 2-3 μ latum; conidiophori amphigeni, fasciculati, moderate vel dence acervati, effusi in mycelium, simplices vel nonnumquam ramosi, parietibus lavibus vel irregularibus praediti, stromati solutoinsidentec, septati, 58-106μ longi, 2.8-3.8 μ lati, pluris ceptati, fusce colarati, cicatricibus minutis sed distinctis; conidia, fusoidia, ali quantulum curvata in medio, apice acute fastigate, 7-36μ longa ut pluri mum 1-6 septata (saepipius 2-4 septata), 28μ longa, 4-8μ lata.

Habitat in follis *Grewia asiatica* Linn.

The type specimen deposited in the Herbarium of the Government Agricultural College, Kanpur and of the Indian Agricultural Research Institute, New Delhi.

The writers are thankful to the Director, Royal Botanical Gardens, Kew, for the help rendered in the identification of the fungus. They are also grateful to Rev. Father Dr. H. Santapau, S. J., Head of the Department of Microbiology. St. Xavier's College, Bombay, for his kindness in translating the diagnosis of the new species into Latin.

Government Agricultural College,
Kanpur

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ADDITIONS TO FUNGI OF BOMBAY—II

A new *Dasturella* on *Boswellia serrata* Roxb.

By

M. K. PATEL, M.M. PAYAK AND N. B. KULKARNI

(Accepted for publication June 29, 1951)

On Vetal hill near Poona, leaves of *Boswellia serrata*—a member of Burseraceæ and a valuable resin yielding and forage tree, were found severely infected by a rust during November-December, 1950. Only uredia and telia have been found. Collections made early in the season showed telia occasionally developing within old uredia. Immature telia developing separately and while still covered with the epidermis can be easily mistaken to belong to the genus *Angiopsora*. However, mature collections reveal that the rust is a species of *Dasturella*. *Dasturella divina* (Syd.) Mundkur and Kheswalla[1], *D. bambusina* Mundkur and Kheswalla[1], and *D. grewiæ* (Pat. and Har.) Thirumalachar[2], are the three known species of the genus. The telia of this rust are somewhat similar to the last mentioned species. It constitutes a fourth species and the name *Dasturella boswelliæ* is proposed. Type is deposited in *Herb. Crypt. Ind. Orient.* and in the Herbarium of the Plant Pathologist, College of Agriculture, Poona.

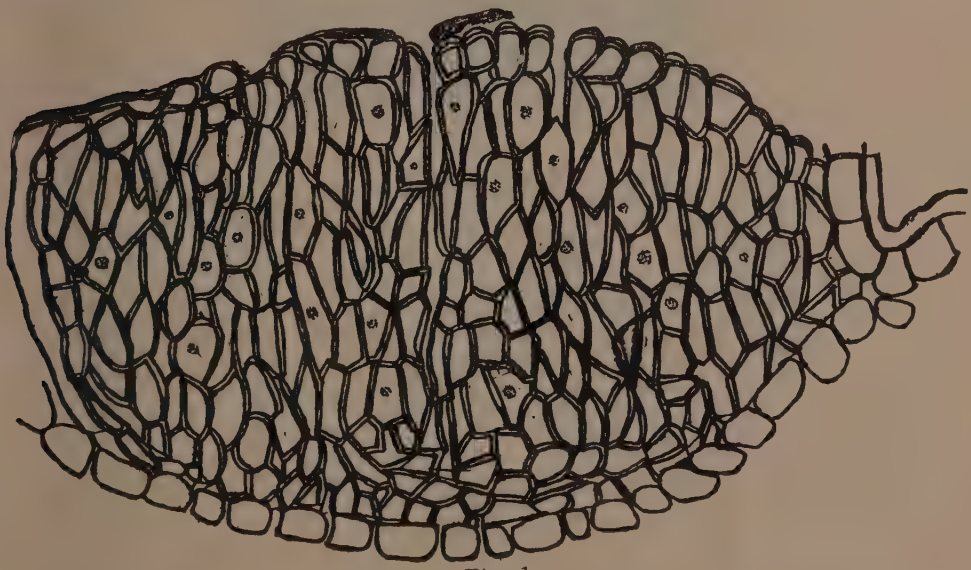

Fig. 1

1. Mundkur, B. B. and Kheswalla, K. F. (1943)—*Dasturella* a new genue of Uredinales *Mycologia* **35** : 201-206
2. Thirumalachar, M. J. (1946)—Notes on three South African Rusts. *Bull. Torrey Bot. Club*, **73** : 346-350

Fig. 2

Dasturella boswelliae Patel, Payak & Kulkarni sp. nov.

Infection spots on the leaves, brownish black, angular, minute and gregarious (Fig. 2). Uredia mostly hypophyllous, minute, reddish brown, paraphysate, paraphyses hyaline, clavate, marginal and intermixed ; the marginal ones slightly incurved and borne on a hyphoid peridium. Urediospores hyaline, obovate to ellipsoidal, 19-32 x 12-17 μ, minutely echinulate, germ pores obscure. Telia (Fig. 1) hypophyllous, subepidermal, mostly developing within old uredia ; telia in compact chains at the beginning, later becoming erumpent, and protruding above the epidermis to produce umbonate to flabelliform crusts 150-290 μ broad, and 76-130 μ high, often coalescent with each other ; teliospores rectangular to polygonal, reddish brown, thick walled, 10-18 spores in each vertical chain, measuring 11-33 x 6-15 μ.

Hab. on the leaves of *Boswellia serrata* Roxb. collected at Poona on 15th November, 1950. (*Type*)

Dasturella boswelliae Patel, Payak & Kulkarni, sp. nov.
Infectionis maculæ in foliis, brunneo-nigræ, angularia, minutæ atque gregariæ.

Uredia ut plurimum hypophylla, minuta, rubro-brunnea, 120-150 μ diam., paraphysata ; paraphyses hyalinæ, clavatæ, marginales atque intermixæ ; paraphyses marginales tenuiter incurvatæ, atque insidentes peridio hypoideo. Uredosporæ hyalinæ, obovatae vel ellipsoideæ, 19-32 x 12-17μ, minute echinulatæ, germinations poris obscuris. Telia hypophylla, subepidermalia, ut plurimum evoluta intra uredia vetera ; telia initiocompacte concatenata, demum erumpentia atque emergentia ex epidermate atque efformantia crustas umbonatas vel flabelliformes, 150-290 μ latas, 76-130 μ altas, saepe inter se coalescentes. Teliosporæ rectangulares vel polygonales, rubro-brunneæ, crassis parietibus ornatæ, 10-18 sporæ in singulis catenis verticalibus, magnit. 11-33 x 6-15 μ.

Habitat in foliis *Boswelliae serratae* Roxb. ; typus lectus in urbe Poona, mense novembri 1950.

We are grateful to Dr. M. J. Thirumalachar for help in determination of the rust and Rev. Father H. Santapau S. J., Head, Department of Biology, St. Xavier's College, Bombay, for furnishing the Latin diagnosis.

Plant Pathological Laboratory

College of Agriculture
Poona

EXLPANATION OF FIGURES

Fig. 1 Erumpent telial column x 450

Fig. 2 Photograph of infected leaves showing angular spots (slightly reduced)

NOMENCLATURE OF BACTERIAL PLANT PATHOGENS

By

M. K. PATEL AND Y.S. KULKARNI

(Accepted for publication June 30, 1951)

Ever since the discovery by Burrill in 1878 that bacteria can cause diseases in plants and erection of the species *Micrococcus amylovorus* for the pathogen inciting fire blight of apples and pears, about 250 phytopathogenic bacteria have been investigated. To accommodate these plant pathogens, the workers either adopted new nomenclature or followed the older ones and in doing so, there has been created considerable confusion and discord. Smith (1905), for instance, noted that Migula's classification primarily meant to accommodate bacteria other than those parasitic on plants, did not meet the requirements of the Plant Bacteriologists and in proposing his own system of classification, erected a new genus *Aplanobacter* for non-motile bacteria. Lehmann & Neumann (1897-1927), Dowson (1939), Elliott (1943), Waldee (1945), Bergey *et al* (1948) and Magrou and Prevot (1948) have proposed different systems of classifications, but none has been universally accepted though each one has its merits.

The progress in bacterial classification has come about largely as a result of the work of specialists in particular groups. For example, Ford studied aerobic spore-forming organisms ; Weldon and Levine devoted their attention to the bacteria inhabiting the intestine ; acetic acid bacteria were studied by Hayer *et al* ; Hucker studied the cocci ; spore forming animal pathogens were studied by an English Commission : the Actinomycetes by Waksman and the root nodule organisms by Fred *et al.*

While the pathogenic and cultural aspects of the plant parasitic bacteria have been investigated intensively and monographed by Bergey *et al*, Dowson, Elliott, Waldee and others, it must be admitted that the same attention has not been paid to evolve a natural system of their classification.

Eubacteriales as at present understood comprise of 13 families which are separated from one another mostly on the basis of their physiological rather than biochemical characters alone. For example, the Enterobacteriaceæ consist of forms which occur in intestinal tracts ; Corynebacteriaceæ, Parvobacteriaceæ and Neisseriaceæ are mainly animal pathogens ; Lactobacteriaceæ are found in milk and its products ; Rhizobiaceæ are principally symbiotic organisms ; Nitrobacteriaceæ reduce or oxidise nitrogenous compounds ; Bacteriaceæ are heterogenous organisms ; Azotobacteriaceæ are non-symbiotic organisms and Bacillaceæ are spore-formers.

It will be seen from the above account that the principal criterion for the classification of Eubacteriales is their physiological behaviour, the morphological and biochemical activities being largely similar as indicated

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1 Bergy, D. H. *et al* (1948). Manual of Determinative Bacteriology, 6th ed.

in Table I, from which it is clear that the plant pathogenic bacteria as most others are short or long rods; generally motile and gram-negative; non-spore formers; not acid-fast; aerobic; methyl red, Voges-Proskauer and indol negative; hydrolyse gelatin, casein and a few starch; ferment dextrose but do not attack cellulose. Above all, they incite diseases in plants only.

If a new bacterium is taken for identification without its source being known, the soil bacteriologist will naturally think it belongs to Nitrobacteriaceæ, Azotobacteriaceæ or Rhizobiaceæ; public health man to Enterobacteriaceæ; medical men to Corynebacteriaceæ or Parvacteriaceæ and a dairy man to Lactobacteriaceæ. The plant bacteriologist, on the other hand, will have to look to genera in different families of Eubacteriales to accommodate the organism under study. It will be an impossible task for an investigator to state the family or genus of a particular bacterium on the basis of any one or a combination of morphological and biochemical characters, unless the source or habitat from which the organism was isolated is known. The inclusion of bacterial organism inciting plant diseases in genera (*Pseudomonas*, *Corynebacterium* and *Bacterium*) and families Pseudomonadaceæ, Rhizobiaceæ, Enterobacteriaceae, Bacteriaceae and Corynebacteriaceae which have been set apart to include animal pathogens and saprophytes, having totally different physiological behaviour, seems therefore to be very incongruous. Further, the names of genera like *Xanthomonas*, *Agrobacterium*, *Pectobacterium*, *Erwinia* and *Phytobacterium* are exclusively used in naming plant pathogens and have not been employed in medical, veterinary or dairy bacteriology. Even though there are numerous yellow pigment producing bacteria among the animal pathogens and soil and water bacterial groups, none is referred to *Xanthomonas*, a genus erected only for plant bacteria. The different plant pathogenic bacteria may incite mild or severe symptoms on their suscepts, but the phytopathogenicity is an important character in separating them as a natural group. The science of phytopathogenic bacteriology is by now quite old and certainly deserves recognition rather than its members finding shelter under different families and also forming sub-sections of genera as in *Pseudomonas*, *Bacterium* and *Corynebacterium*. It is, therefore, fitting to allocate a separate family for their accommodation for which the name Phytobaeteriaceae is proposed. Consequently, when any genus is referred to Phytobacteriaceae, one can be certain that it refers to plant pathogens alone. Species belonging to *Pseudomonas*, *Corynebacterium* and *Bacterium* cannot be expected to be parasitic on totally different substrata like the plants and animals. *Pseudomonas aeruginosa* (Schroet) Migula claimed to occur in both plant and animal substrata is chiefly a saprophyte.

In establishing different genera comprising the family Phytobacteriaceae, the general principle adopted by Burkholder, Waldee, Dowson, Conn, Bergey and others, that the genera be broadly grouped on the basis of symptoms produced in addition to the morphological and biochemical characters is followed. Even though the severity of the symptoms incited by the pathogen is conditioned by the availability of suitable predisposing conditions and susceptible host, the general symptomatology considered in a broad sense like leaf spot and stripe, wilt, soft rot, hypertrophy and blight etc. seem to be characteristic of particular genera of Phytobacteriaceae.

It is manifest that this classification is a natural one since it facilitates quick diagnosis and brings generally to mind the type of symptoms which each genus produces besides showing relationships at a glance, and thus proving the change more convenient and useful, even to a beginner. The description of the family and the genera now follows :—

PHYTOBACTERIACEAE fam. nov.

Organisms yellow, white, fluorescent or variant; short or long rods; motile with mono-lopho or peritrichiate flagella or non-motile. Mostly gram-negative : a small number gram-positive. No endospores. Capsulated or otherwise ; not acid-fast ; not attacking cellulose ; indol production nil or slight : aerobic ; dextrose fermented with or without gas. Optimum temperature for growth 20-30°C., maximum 37°C. with thermal death point never exceeding 52°C. Plant pathogens causing leaf-spot or cankar, soft-rot, gall, wilt and blight.

In considering the different genera under Phytobacteriaceae the following points need careful consideration :

(*a*) The genus *Xanthomonas* Dowson which includes yellow pigment producing phytopathogenic bacteria should be considered valid since it incites necrosis in leaves, stems and petioles. It is proposed that the genus be transferred from Pseudomonadaceae to the new family Phytobacteriaceae.

(*b*) Magrou and Prevot (1948) have recently split the genus *Pseudomonas* Dowson into two genera *viz.* *Phytobacterium* for white phytopathogenic organisms consisting of 22 species and the genus *Pseudomonas* for the rest of the phytopathogenic organisms originally placed under *Pseudomonas* by Dowson. On closer examination, it is found that only 16 of these are true to the genus *Phytobacterium* described by the authors, 3 are yellow and have already been transferred to *Xanthomonas*, 1 is a repetition and 2 are obsolete.

(*c*) Under the genus *Pseudomanas* as conceived by Magrou and Prevot, there remain 62 green fluorescent pigment producing phytopathogenic organisms. The genus *Pseudomonas* as at present conceived includes a large number of saprophytes found in pus, soil, water and other sub-strata so that it seems untenable to include phytopathogenic bacteria under the same genus. In order to remove this confusion, therefore, it is proposed to transfer the phytopathogenic organisms now placed under this genus to a new genus to be called **Chlorobacter,** a fitting name since the species produce green fluorescence.

Chlorobacter gen. nov.

Syn. *Pseudomonas* Migula emend Dowson in *Trans. Brit. Mycol. Soc.* 26 : 4, 1943 and Magrou and Prevot in *Compt. Rend, Acad. Sci.* (Paris) 226 : 1229-1230, 1948

Organisms producing green fluorescent water soluble pigment ; one to several polar flagella ; gram-negative ; mostly entering the host through natural openings ; gelatin generally liquefied ; starch hydrolysed ; non-lipolytic ; acid but no gas in several mono and disaccharides ; salicin not fermented ; lactose fermented ; M. R. and V. P. tests negative. Plant

pathogens primarily inciting spot and canker of leaves, stems, fruits and branches ; rarely blight.

Type species : *Chlorobacter syringae* (van Hall) comb. nov.

(d) The genus *Aplanobacter* was founded by Smith (1905) for *A. michiganense* inciting bacterial canker in tomato. The lack of any flagella for the bacterium was stressed as an important character in distinguishing the genus, while the gram-positive nature of the organism and other characters were then given secondary importance only. Four more species were added to the genus, viz., *A. insidiosum* McCulloch, *A. rathayi* Sm., *A. sepedonicum* and *A. agropyri* O'gara. Since the mere atrichous character of the bacterium was not significant enough by itself to separate a genus, all the later investigators rejected it and placed the species under *Bacterium* and later under *Phytomonas*. Dowson (1942) considering the non-motile and gram-positive nature of the bacteria as main characters placed these important pathogens under *Corynebacterium* ignoring the fact that this genus was erected to accommodate a serious animal pathogen (*C. diphtheriae*). The genus *Aplanobacter* erected to include only phytopathogenic bacteria with gram positive and non-motile characters needs to be reinstated as a valid genus and cor *ryncebacterium* should refer only to animal pathogens and saprophytic bacteria for which it was originally erected by Lehmann and Neumann. These important plant pathogens are considered to be valid species of *Aplanobacter*.

Recently Magrou and Prevot (1948) have suggested that the genus *Aplanobacter* should include non-motile, non-spore forming, gram negatives whose type species is *A. stewartii*. The original conception of Smith of *Aplanobacter* as emended by the authors includes 6 non-motile, gram positive phytopathogens. *A. stewartii* as suggested by Magrou and Prevot has already been transferred to *Xanthomonas stewartii* because of its yellow pigment, motility and gram negative characters. One gram positive organism in the genus *Chlorobacter* and one in *Xanthomonas* as compared to a total of 144 gram negative phytopathogenic organisms (table 1) may on further investigation be found to be gram negative and motile. It is, therefore, proposed that the idea of Magrou and Prevot to transfer the gram positive, non-motile, phytopathogenic organisms to a separate group should be rejected, thus retaining the original genus *Aplanobacter* for gram positive, non-motile, phytopathogenic organisms.

Aplanobacter Smith in *Bacieria in Relation to Plant Diseases*. Vol. 1 p. 171, 1905—emend Patel and Kulkarni

Syn. *Corynebacterium* sensu Dowson in *Trans. Brit. Mycol. Soc.* 25 : 311, 1942

Organisms white or producing different shades of colours ; club shaped ; non-motile ; gram-positive ; starch not hydrolysed ; little or no lipolytic activity ; lactose fermented ; no gas ; M. R. and V. P. negative. Plant pathogens causing wilt.

Type species : *Aplanobacter michiganense* Smith

(e) The genus *Agrobacterium* proposed by Conn for including *A. tumefaciens* is considered in the present paper as a good genus since its members incite tumour, hairy root and other malformations. It is, therefore, proper to transfer this genus to Phytobacteriaceae from Rhizobiaceae, which now should include only the nitrogen fixing symbiotic bacteria.

(f) The genera *Erwinia* and *Pectobacterium* as cited by Bergey *et al* include coloniform, peritrichiate, gram-negative bacteria causing blight and soft rot of fruit and vegetables. Waldee separated the genus *Pectobacterium* from *Erwinia* since the former differed by its ability to attack pectin and produce gas in several cases. The retention of the name *Erwinia* which includes organisms causing blight is, however, most fitting since the foundation of phytopathogenic bacteriology was laid by Erwin F. Smith for whom it was named. The question of *E. atroseptica* inciting the blackleg of potato could not be raised since it is capable of attacking the middle lamella whether it was freshly isolated or grown for a prolonged period on certain media and it should, therefore, be transferred to the genus *Pectobacterium.*

(g) *Erwinia* Winslow in *Manual of Determinatiuc Bacteriolngy* 1st ed. p. 404, 1923-emand Patel & Kulkarni.

Organisms white ; peritrichiate ; gram-negative ; starch attack variable ; no lipolytic activity ; pectin not attacked ; lactose not fermented ; no gas ; M. R. and V. P. tests negative. Plant pathogens causing blight.

Type species : *Erwinia amylovora* (Burrill) Winslow *et al*

Two keys (detailed and simple) of Phytobacteriaceae for ready reference and a list of some organisms forming new combinations as per suggestions made in this paper are also given at the end of this paper.

SUMMARY

Reasons for the creation of the family Phytobacteriaceae to include all phytopathogenic bacteria are given. The new family includes 7 genera primarily based on the types of symptoms produced.

Chlorobacter nov. gen. for leaf-spot producing, green fluorescent organisms is recommended. The genus *Aplanobacter* is amended to include gram positive, non-motile, wilt producing organisms. The genus *Erwinia* is also retained for peritrichous plant pathogens not attacking pectin.

The authors are greatly indebted to Drs. B. B. Mundkur and M. J. Thirumalachar for carefully going through the manuscript several times and suggesting important alterations.

The authors also wish to thank Rev. Father H. Santapau for suggestions.

Plant Pathological Laboratory
College of Agriculture
Poona 5

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A list of some of the several bacterial plant patbogens which will now form new combinations

CHLOROBACTER nov. gen.

C. aceris (Ark)
C. aleuritidis (McCulloch and Demaree)
C. angulatum (Fromme and Murray)
C. apii (Jagger)
C. aptatum (Brown and Jamieson)
C. barkeri (Berridge)
C. bowlesii (Lewis and Watson)
C. calendulae (Takimoto)
C. cichorii (Swingle)
C. coronafaciens (Elliott)
C. delphinii (Sm.)
C. erodii (Lewis)
C. glycineum (Coerper)
C. intybi (Swingle)
C. lachrymans (Smith and Bryan)
C. lapsum (Ark)
C. marginale (Brown)
C. marginatum (McCulloch)
C. martyniae (Elliott)
C. medicaginis (Sackett)
C. mori (Boyer and Lambert)
C. papulans (Rose)
C. pisi (Sackett)
C. primulae (Ark and Gardner)
C. sesami (Malkoff)
C. setariae (Okabe)
C. striafaciens (Elliott)
C. syringae (Van Hall)
C, tabacum (Wolf and Foster)
C. viciae (Uyeda)
C. viridiflavum (Burkholder)
C. viridilividum (Brown)
C. xanthochlorum (Schuster)

PHYTOBACTERIUM Magrou and Prevot

P. mangiferae-indicae (Patel, Moniz and Kulkarni)
P. solanacearum (Sm.)
P. vitis-woodrowii (Patel and Kulkarni)

PECTOBACTERIUM Waldee

ananas (Serrano)
atrosepticum (Van Hall)
betivorum (Takimoto)
cytolyticum (Chester)
rhapontici (Millard)

TABLE I

Morphological and biochemical characters of some bacterial organisms

Families	Genera	Shape	Gram stain		Motility			Gelatin liquefaction		Nitrite production		Gas production	Hydrolysis of starch		No. of spp. considered	Remarks
					Polar	Peritrichiate	Nil									
			−	+				+	−	+	−	+	+	−		
Phytobacteriaceae	Chlorobacter		62	1	62	...	2	52	11	12	44	...	18	28	67	Spot inciting, green
	Phytobacterium		16	...	16	...	...	7	9	6	8	...	7	6	16	,, ,, white
	Xanthomonas		44	1	46	.	...	39	3	14	29	...	28	5	49	,, ,, yellow
	Agrobacterium		6	...	6	...	...	2	3	3	2	...	...	5	6	Gall ,,
	Erwinia		6	...	...	6	...	4	2	4	2	...	1	2	6	Blight ,,
	Pectobacterium		14	...	..	14	...	11	3	11	3	8	3	8	14	Rot ,,
	Aplanobacter		...	5	...	..	6	4	2	3	2		3	1	6	Wilt ,,
Pseudomonadaceae	Pseudomonas (white)		31	1	29	..	3	16	10	17	15	...	3	4	64	Soil
	Pseudomonas (yellow)		15	.	14	...	1	7	6	9	6	...	4	3	15	,,
Bacteriaceae	Bacterium Aerobacterium Flavobacterium	RODS	27	27	18	7	34	30	21	14	25	...	17	12	53	Soil, water
	Bacterium		3	...	2	...	1	...	3	1	1	...	...	3	3	Transferred to *Xanthomonas*
Enterobacteriaceae	Escherichia		6	...	...	2	2	3	3	6	...	3	...	...	6	Colon
	Serratia		5	...	...	5	..	5	..	3	...	...	.	...	5	
Rhizobiaceae	Rhizobium		6	...	...	6	...	...	...	...	...	...	...	...	6	Symbiotic
	Chromobacterium		3	...	...	3	..	3	...	2	1	...	...	...	3	
Corynebacteriaceae	Corynebacterium		1	2	2		1	.	2	2	1	...		3	19	Animal
	Listeria		.	.	.	...	...	.	1	.	.			..	1	pathogens, Dairy,
	Erysipelothrix		..	2	.	..	2	.	2	...	1	...	..	...	3	Water, Soil
Nitrobacteriaceae	Nitrosomonas		2	2	3	..	2	...	...	...	...	...	...	...	14	
	Nitrocystis		1	.	2	...		...	2	...	1		...	...	3	Oxidising NH_3
	Nitrosocystis		3	...	4	...	1	1	...	...	...	...	...	...	5	NO_2 H, S.
			247	64	205	43	74	192	90	112	152	11	85	80	364	

Simple key showing (A) relationships between different nomenclatures

(A)	Motile (Polar)	Motile (Polar) and Non-motile	Non-Motile	Non-motile and Motile (Peritrichous)	(Motile) (Peritrichous)		Authority
	Pseudomonas	—	Bacterium	—	Bacillus		Migula (1895-1900)
	Bacterium	—	Aplanobacter	—	Bacillus		Smith (1905)
	—	Phytomonas	—	—	Erwinia		S. A. B. (1923)
	Pseudomonas Xanthomonas Phytobacterium	—	Corynebacterium	Bacterium	—		Dowson (1939, 1942)
	Pseudomonas Xanthomonas Agrobacterium	—	Aplanobacter	—	Erwinia		Magrou & Prevot 1948)
	Pseudomonas Xanthomonas Agrobacterium	—	Corynebacterium	—	Pectobacterium	Erwinia	S. A. B. (1948)
	Chlorobacter Phytobacterium Xanthomonas Agrobacterium	—	Aplanobacter	—	Pectobacterium	Erwinia	Patel & Kulkarni (1951)

Simple key showing (B) relationship between genera of Phytobacteriaceae

(B)	Xanthomonas	Phytobacterium	Chlorobacter	Agrobacterium	Aplanobacter	Pectobacterium	Erwinia
Symptoms	Leaf-spot	Leaf-spot	Leaf-spot	Hypertrophy	Wilt	Rot	Blight
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Colour	Yellow	White	Green fluorescent	White	Different shades, gray	White	White
Gram	—	—	—	—	+	—	—
Gas	—	—	—	—	—	±	—
Motility	Polar	Polar	Polar	Polar	—	Peritrichous	Peritrichous
Starch	+	±	±	—	—	—	±
Salicin	No acid	No acid	No acid	Acid			Acid
Lactose	Acid	No acid	Acid	Acid	Acid	Acid	No Acid

Detailed key to the genera of PHYTOBACTERIACEAE Nov. Fam

Yellow, white, fluorescent or variant ; short or long rods; motile with mono-lopho or peritrichiate flagella or non-motile. Mostly gram-negative ; a small number gram-positive. No endospores. Capsulated or otherwise. Not acid fast. Aerobic. Do not attack cellulose. Indole production nil or slight. Dextrose fermented with or without gas. Optimum temperature for growth 20-30°C. Maximum 37°C., Thermal death point 51°C. Strict plant pathogens inciting necrosis, soft rot, gall, wilt and blight.

I. Mostly entering through natural openings. Gram-negative. Motile by polar flagella. Salicin not fermented, acid but not gas from several mono and di-saccharides, M. R. and V. P. tests negative. Plant Pathogens mostly inciting leaf-spot, stripe and canker on leaves, stems, fruits aad branches.

(A) Yellow, water insoluble pigment with 1 or 2 polar flagella, gelatin liquefied, starch hydrolysed, lipolytic, leaf-spot inciting ..... *XANTHOMONAS* Dowson

(B) White with 1 to several polar flagella. Gelatin generally liquefied, starch mostly hydrolysed, non-lipolytic, mainly leaf-spot inciting, rarely blight ..... *PHYTOBACTERIUM* Magrou and Prevot

(C) Same as above except that they produce green fluorecsent water soluble pigment, lactose fermented ..... *CHLOROBACTER* nov. gen.

II. Mostly wound parasites. M. R. and V. P. negative, little or no lipolytic activity. A small number gram-positive. Mostly peritrichiate, some non-motile. Acid with or without gas from mono or di-or polysaccharides. Organisms white or of different shades of colour. Plant pathogens inciting soft rot, gall, fasciation, wilt and blight.

(A) Acid with or without gas from several sugars, gram-negative, peritrichiate, salicin, maltose and lactose fermented, pectin attacked, M. R. and V. P. negative, gelatin attacked, inciting soft rot ..... *PECTOBACTERIUM* Waldee

(B) Gram-negative, peritrichiate, lactose not fermented, pectin not attacked, inciting blight primarily..... .... *ERWINIA* Winslow *et al emend* Patel and Kulkarni

(C) Acid from several sugars, gram-negative, mono or lophotrichous, salicin, maltose and lactose fermented, pectin not attacked, M. R. and V. P. negative, gelatin not attacked, inciting gall or fasciation on root or stem..... .... *AGROBACTERIUM* Conn

(D) Gram-positive, non-motile, lactose fermented, club shaped, with different shades of colours, inciting wilt..... *APLANOBACTER* Smith emend Patel and Kulkarni

THE EFFECT OF TEMPERATURE AND LIGHT ON THE MESOTHETIC REACTION OF JOSTRAIN OATS TO STEM RUST[1]

By

M.K. HINGORANI[2]

(Accepted for publication, July 25, 1951)

It has been observed that during the summer months the reaction of one differential host, Jostrain, to certain races of *Puccinia graminis avenae* Erikss. and Henn. fluctuates beyond the normal limits. This was attributed to variations in environmental conditions in the greenhouse.

Waterhouse (1929) found that, when the temperature in the greenhouse was high, Jostrain oats were completely susceptible to race 1 of the variety *avenae*, whereas they were resistant at low temperatures. The types of infection produced by races 2 and 7 were, however, not significantly changed on any of the hosts.

Gordon (1930,1933) reported the results of a detailed study on the effect of temperature upon the host reactions to eight physiologic races of *Puccinia graminis avenae*. He showed that certain races have a tendency to produce type 1 infection, or even necrotic flecks, on seedling leaves of Jostrain at a temperature of 13° to 16° C., and X type, or even types 3 to 4, at a temperature of 24° to 25°C. He concluded that the variations in temperature that occur commonly in the greenhouse were capable of affecting the infection types in certain race x variety combinations.

Light is also known to affect rust development very strongly. Johnson (1931), in working with some races of *Puccinia graminis tritici*, noticed that the infection type X was variable. He was able to induce the production of infection types ranging from O ; through type X up to type 4 by the same race on the same variety, corresponding with low, intermediate, and high light intensities.

In the present investigation, therefore, the writer attempted to study the effect of temperature and light on the reaction of the differential host, Jostrain, to physiologic races 2, 5, 8 and 10 of *Puccinia graminis avenae*. Races 2 and 5 differ only in their effects on the variety Jostrain, race 2 producing type 4 and race 5 type X (mesothetic reaction). There is a

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2. The author wishes to express his gratitude to Watumull Foundation for the grant of a Fellowship and to Dr. E.C. Stakman, Chief of the Division of Plant Pathology, University of Minnesota, for his guidance in this research.

similar difference between races 8 and 10 on Jostrain, race 8 producing type 4 and race 10 type X.

A single-sorus culture of each of the four races was obtained and the cultures derived from them were used as inoculum throughout the study. Jostrain was inoculated in the seedling stage, kept in a moist chamber for 48 hours, and then shifted to the sections of the greenhouse which were maintained at constant temperatures of 18°, 24° and 30°C. The three light intensities used in the light experiment averaged about 7000(5000-9000), 1000(500-1500), and 250(100-400) foot-candles ; temperature of the greenhouse during the experiment was about 24°C. Data are summarized in Tables I and II.

TABLE I

Effect of temperature on the types of infection produced by races 2, 5, 8 and 10 of Puccinia graminis avenae *on Jostrain oats*

Race	Type of infection produced at temperatures		
	18°C.	24°C.	30°C.
2	X−	4+	4
5	X=	X	3 to 4
8	3+	4+	4
10	X=	X	3 to 4

TABLE II

Effect of light on the types of infection produced by races 2, 5, 8 and 10 of Puccinia graminis avenae *on Jostrain oats*

Race	Type of infection produced at light intensities		
	Low	Medium	High
2	3+	4+	4+
5	X−	X	3
8	3+	4	4+
10	X−	X	3+

Under these conditions (Table 1) temperature definitely influenced the reaction of the differential host to three of the races but scarcely affected its reaction to race 8. The normal infection types were

produced at 25°C. ; races 2 and 8 produced infection type 4+, races 5 and 10 produced infection type X. At 18°C., however, Jostrain was somewhat more resistant to races 2, 5, and 10, the infection type X being produced by all three races. Sporulation of race 8 was reduced at 18°C., but Jostrain was still susceptible to the race. With a high temperature of 30°C., Jostrain became more susceptible to races 5 and 10 and the infection types produced by these races varied from 3 to 4.

Light intensity had no decided effect on the infection type 4 produced by races 2 and 8 on Jostrain. Races 5 and 10, however, produced infection types 3 to 3+ at high intensity of light and type X— at low intensity. Under the intermediate conditions the normal mesothetic reaction was produced.

Thus these results show that infection type X, produced by races 5 and 10 on Jostrain, is more variable than types 3 and 4 under varying temperature and light conditions.

CONCLUSION

The principle effect of temperature on infection types was on type X produced by races 5 and 10 on Jostrain. At 30°C. the sporulation was so abundant that the infection types present were recorded as 3 to 4 ; at 18°C. the sporulation was reduced and the infection type was X=; and at 24° C. typical mesothetic reaction was produced. With race 2, the only change observed was at 18°C. where the sporulation was considerably reduced and the infection types were classified as X—. Race 8, however, did not change at any of these temperatures.

Light also had some effect on the infection type X produced on Jostrain but it was not so pronounced as that of temperature.

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Book Reviews

Leaf Curl Diseases of Cotton. By S.A.J. Tarr (The Commonwealth Mycological Institute, Kew, Surrey), 1951. pp. 55.

This small well got up monograph on the leaf curl disease of cotton published by the Commonwealth Mycological Institute in its new series of publications is a welcome one. Much of the work in progress in the Sudan is clearly summarised and results of similar investigations from other cotton growing countries, notably, Indian work on virus diseases of cotton and their insect vectors, are juxtaposed against the Sudanese work to make a connected story of the leaf curl problem. The Chapter on 'Virus diseases of the Malvaceae other than cotton' contains valuable material on collateral hosts in the same family which act as sources for spread of the cotton leaf curl and tobacco leaf curl viruses. The Chapters on 'Symptomatology' and 'Transmission of cotton leaf curl' are well illustrated, although one can not help remarking that most of the illustrations do not give either the reduction or the magnification, particularly, photomicrographs in Plate X (facing p. 19) of transections of the healthy and diseased leaves of cotton infected with the leaf curl virus. There are other deficiencies, as for instance, the lack of complete title of the works under 'References', and again the omission of some of the important reference books on Plant Viruses like the works of F.C. Bawden. While reading the text one feels that apart from the usefulness of having all the available data on the leaf curl disease on cotton under one cover, these reviews published under the auspices of the C.M.I., are really meant to stimulate new ideas in the reader's mind ; this obvious advantage the author has not fully utilised, although in patches he has indicated possibilities of future fruitous researches in certain unexplored fields.

It is to be hoped that this monograph would stimulate more work in cotton growing countries of the world, particularly, in the fascinating field of isolation and purification of the cotton leaf curl virus and the study of its *in vitro* and *in vivo* properties. This, to the reviewer, appears long overdue considering the very wide geographical distribution enjoyed by the virus and the economic loss the cotton crop suffers year after year. Fundamental work is all the more indicated since the author has mentioned in Chapter on 'Inter-transmissibility of the cotton leaf curl virus' that there are many strains of *Gossypium* (*G. barbadense* and *G. hirsutum*) showing a virus disease spectrum, as it were, varying from high resistance to high susceptibility. There is, however, no indication of the presence of masked strains of the virus being present in the cotton leaf curl. Work, therefore, on isolation and purification of this virus, and possibly strains of it, would bring to the forefront the need for even an attempt at a serological classification of the cotton leaf curl virus.

This book has admirably fulfilled a gap that existed in literature on some virus diseases of the cotton and has brought together a mass of very valuable data which, as the author points out, lay scattered in reports and journals hitherto.

T. S. S.

Manual of Rice Diseases by G. Watts Padwick, Viii 198 pages, Commonwealth Mycological Institute, Kew, Surrey, 1950.

In this Manual the author has succeeded in bringing together under one cover all the available information on the diseases affecting the rice plant. The Manual is divided into 3 parts. Part I deals with the diseases of parasitic origin and Part II with diseases due to non parasitic causes. Part III is an annotated check list of fungi recorded on rice. There are 5 chapters in Part I dealing respectively with the diseases of the foliage, stem and leaf-sheath, seedling blights and foot-rots, diseases of the grain and inflorescence and those caused by nematodes. There are three chapters in Part II dealing respectively with the diseases due to unfavourable soil conditions, deficiencies and viruses.

Some of the diseases of rice like blast, brown-spot, stack-burn, stem-rot, bakanae, ufra and stunt which have been very extensively investigated, are described in considerable detail. The history of the diseases, the symptoms they cause, the causal organisms and their biology, the nature of resistance and physiologic races, if any, and the control measures, are fully discussed, but this sequence has not been uniformly followed in all cases. There are good bibliographies after each disease, which will help investigators in referring to original literature. Where the diseases have not been properly investigated, the accounts are necessarily brief. Under stem-rot the author has given the symptoms recorded by Butler as typical of the disease in India. It is well known that in a rice field there are usually a large number of heploid plants which are characterized by an excessive number of tillers which tend to remain green and sterile.

Both Butler and Shaw erroneously considered this condition as having been caused by the fungus responsible for stem-rot. In a series of experiments which the reviewer did some years ago he noted that there were severed isolates of this fungus which under laboratory and field conditions failed to produce the typical stem-rot symptoms described by Cattaneo, Miyake, Tisdale, Tullis and others, or those detailed by Butler and Shaw. He was able to detect the sclerotia of the fungus even in very healthy plants. However, Luthra and his colleagues were able to record the disease in the Punjab which showed the symptoms associated with stem-rot. From such plants they not only isolated the precise fungus but proved its pathogenicity also. Recently rice plants attacked by this disease and showing typical stem-rot symptoms, have also been recorded in the Tanjore delta of the Madras State, proving that these are parasitic strains in India. The emphasis placed by Butler and Shaw on partial sterility and excessive tillering is thus an error and the author would have done well to clarify this rather than perpetuate the mistake which they had made.

The causal organism of the ufra disease has been renamed *Ditylenchus angustus* but it is not clear why the author refers to it on page v of the Preface as *Anguillullina angusta*. The virus diseases of the rice plant are placed in part II which deals with non-parasitic diseases. This is surprising for viruses are very good parasites, transmitted from plant to plant by insect vectors. They cannot be considered as non-parasitic because the viruses are ordinarily invisible.

In the annotated check-list of fungi recorded on rice, the author has transferred two fungi, *viz. Phaeosphmria cattane* and *Phaeosphaeria oryzœ*, to the genus *Trematosphærella* but it is not clear if the name *Trematosphærella* itself is correct, even though it pre-dates *Phœosphœria*. The fungus named by Saccardo in 1916 as *Phyllosticta glumarum* has been renamed *Phyllosticta oryzina* as the former name was occupied but it would have been advisible if the author had compared this fungus with Hara's *Phyllosticta oryzaecola*, the existence of which he does not seem to know. In Italy *Coniothyrium cattanei* is known to cause spots on leaves and sheaths of the rice plants. It has not been mentioned by the author. An alga *Hydrodictyon reticulatum* is known to attack roots of young rice plants in Italy. This has apparently escaped the author's notice.

B. B. M.

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